THE AMERICAN JOURNAL
OF PATHOLOGY

Official Publication of
The American Association of Pathologists and Bacteriologists

VOLUME XXIII

1947

CONTENTS OF VOLUME XXIII

1947

SEPTEMBER, 1947. NUMBER 5

NOVEMBER, 1947. NUMBER 6

THE AMERICAN ASSOCIATION OF PATHOLOGISTS AND BACTERIOLOGISTS

Forty-Fourth Annual Meeting, University
of Illinois, Chicago, Illinois

May Sixteenth and Seventeenth, 1947

PRESIDENT FORBUS IN THE CHAIR

BUSINESS MEETING

May Sixteenth, 1947

For the Council, the Secretary announced the following actions:
Election of new members

Charles P. Baker, Oakland, Calif.
Parker R. Beamer, St. Louis
Warren A. Bennett, Washington
William G. Bernhard, Summit, N.J.
Herman T. Blumenthal, Louisville
Warren L. Bostick, San Francisco
William H. Carnes, Baltimore
Jacob Churg, Paterson, N.J.
Jose Curiel, Mexico City
William L. Donohue, Toronto
Carl E. Duffy, Grosse Pointe, Mich.
Thelma B. Dunn, Bethesda, Md.
Patrick J. Fitzgerald, New York
Alfred Golden, Memphis
Clinton V. Hawn, Cooperstown, N.Y.
Elwyn L. Heller, Pittsburgh
Benjamin Highman, Silver Spring, Md.
Howard C. Hopps, Oklahoma City

Pao-chang Hou, Chengtu, Szechuan, China
Lalla Iverson, Durham, N.C.
Alfred G. Karlson, Rochester, Minn.
B. H. Kean, New York
Joseph F. Kuzma, Wauwatosa, Wis.
Thomas C. Laipply, Chicago
Frederick H. Lamp, Davenport, Iowa
Raffaele Lattes, New York
Stuart Lindsay, San Francisco
Ernst Loeffler, Chicago
Alfred M. Lucas, East Lansing, Mich.
Mark E. Maun, Detroit
Frank W. McKee, Rochester, N.Y.
Joseph F. A. McManus, Birmingham, Ala.
George Milles, Chicago
John Edgar Morison, Belfast, Ireland

Richard E. Olsen, Pontiac, Mich.
Lawrence Parsons, Reno, Nev.
Machteld E. Sano, Philadelphia
Edward C. H. Schmidt, Kansas City, Mo.
Ruell A. Sloan, Arlington, Va.

Edward B. Smith, Narberth, Pa.
Cyril Solomon, New York
Paul B. Szanto, Chicago
John L. Tullis, Bethesda, Md.
Lyle A. Weed, Rochester, Minn.
O. J. Wollenman, Jr., McKinney, Texas

Reinstatement to membership of Drs. Istvan A. Gaspar, Hugh G. Grady, and Preston Kyes.

Acceptance, with regret, of the resignations of Drs. James Miller, Max Pinner, and George Shanks.

The Council announced, with deep regret, the deaths of Drs. George T. Caldwell, Mortimer Cohn, L. U. Gardner, W. P. Larson, Emanuel Libman, Ward H. MacNeal, and H. E. Robertson.

Upon nomination of the Council, the Association voted to elect the following officers:

President	MALCOLM H. SOULE
Vice-President	E. W. GOODPASTURE
Secretary	HOWARD T. KARSNER
Treasurer	ALAN R. MORITZ
Incoming Member of Council	ROBERT A. MOORE

The Council announced that it had accepted the invitation of Dr. Virgil H. Moon to hold the next annual meeting of the Association at the Jefferson Medical College, Philadelphia, and that the meetings will be held on the Friday and Saturday preceding the meetings of the Federation of American Societies for Experimental Biology.

The Council announced that the topic for the symposium in 1948 will be "Diseases of Bones" and that Dr. Henry L. Jaffe will act as referee.

For the Council, the Secretary announced the re-election of Dr. Carl V. Weller as Editor-in-Chief of *The American Journal of Pathology* for the term of seven years; the re-election of Dr. Malcolm H. Soule as Assistant Editor of *The American Journal of Pathology* for the term of one year; the election of Dr. R. Philip Custer to the Editorial Board of *The American Journal of Pathology* for the term of seven years, to succeed Dr. J. Harold Austin whose term has expired.

The Secretary read a notice from Captain G. B. Ribble, U.S.N., in reference to Naval Reserve Medical Officers.

The Secretary read a letter from Dr. O. J. Pollak inviting attendance

ADNEXAL CARCINOMA OF THE SKIN *

NATHAN CHANDLER FOOT, M.D.

(*From the Department of Surgical Pathology of the Cornell University Medical College and the New York Hospital, New York, N.Y.*)

Much confusion exists concerning the classification and histogenesis of that group of tumors arising from the epidermis and its adnexa generally known as "basal cell epithelioma." Some of its members are noncancerous, while others are frankly, though not intensely, malignant. In his textbook on dermatological pathology, McCarthy [1] groups these under the heading "Basal Cell Epithelioma" and recognizes four types, all of which he considers benign. They are: (1) growths developing from groups of cells located beneath the epidermis or growing downward into the cutis as prolongations from it; (2) epithelioma adenoides cysticum of Brooke; (3) small, multiple, walnut-sized tumors appearing in groups and having a widespread distribution (morphologically intermediate between groups 1 and 2); and (4) cylindroma. Discussing group 3, he indicates that its members arise in a multicentric fashion and exhibit a definitely cystic architecture which is fundamentally that of "basal cell epithelioma"; that is to say, it presents masses of pleomorphic cells packed into spheroidal cores that are enclosed by a membrane of radially arranged basal cells. Many of these masses undergo cystic degeneration.

Such a classification is purely morphological and the student or young pathologist reading it will find himself much confused when he turns to other books or articles and discovers the "cylindroma" referred to as "Brooke's tumor," or as "hydradenoma solidum." The tumors of McCarthy's groups 1 and 3 are called "basal cell carcinoma" or "hair matrix carcinoma" according to the views of the particular authority consulted. Thus it is obvious that the student's confusion is quite natural and one must infer that our knowledge of these growths is equally confused, as well as faulty. It is the purpose of this paper to attempt to bring some order out of the existing muddle.

* Received for publication, December 28, 1945.

HISTORICAL REVIEW

At the risk of being prosaic it will be necessary to trace the history of the theories as to "basal cell tumor." It was first christened "the gland-like carcinoma of the superficial epithelium, carcinoma epitheliale adenoides," by Krompecher [2] in 1900; in 1903 he published a monograph entitled "Der Basalzellenkrebs," and expanded his conception of an origin in basal cells of the epidermis to include as well those of the epidermoid mucosa of the oral cavity, nasal mucosa, larynx, and similar regions.[3] He recognized four types of growth; solid, pouch-like downgrowths from the epidermis; epithelial masses containing cysts; glandular forms composed of interlacing bands of epithelium; and nests of epithelium that produced parakeratotic pearls. He considered origin in hair follicles, sebaceous glands and sudoriparous glands, which is interesting in view of what is to follow in this paper. Unfortunately, in the later monographs, he condensed these possibilities into what might be considered an oversimplified probability: origin of all these tumors, including those of epidermoid and schneiderian mucous membranes, from the basal layer of germinal cells.

There the matter has rested for almost 45 years, and the vast majority of pathologists and dermatologists speak of basal cell carcinoma as an undisputed entity. Holding to this view, they are forced to explain certain incongruities in the behavior of the tumor. Why should it be only mildly malignant if it be derived from the least differentiated and the most primitive cell of the epidermis? The apparently better differentiated epidermoid or squamous cell carcinoma is more malignant; why is that the case? Again, why is the basal cell carcinoma a trivial affair in the skin, while it is notoriously malignant when it arises from the epidermoid mucosa of the oral cavity or esophagus? Lastly, why should this basal cell tumor take on a regularly characteristic architecture that bears no close resemblance to epidermis and often consists simply of two types of cell? This latter form is pathognomonic of the so-called "basal cell carcinoma." One is impelled to ask, in the vernacular, "how do basal cells manage to get that way?"

In 1910 Mallory [4] demonstrated fine longitudinal fibrils in the cells of the tumor which were identical in their appearance with similar fibrils in cells of embryonal hair follicles and quite unlike the intracellular fibrils of the prickle cells of the epidermis, the normal first-born offspring of the basal cell. From this he deduced that the tumors arose from the cells of hair matrices, sebaceous and sudoriparous glands. He believed that the basal cells of the epidermis were early forms of prickle cells and had the same type of intracellular fibrils, filaments that run in all directions and are intercellular as well as intracellular. Unfor-

tunately, his findings were limited to these fibrils and his report constituted a small part of a lecture on "Recent Progress in the Microscopic Anatomy and Differentiation of Cancer," so that his theory on "hair matrix carcinoma" was buried in an article, the title of which gave no clue to its presence. In 1914 he published his "Principles of Pathologic Histology," and in it he restated the theory and mentioned the tumor as a "hair matrix carcinoma" which had its origin in the hair matrices. He did not document this opinion in any way other than to call attention to the fibrils mentioned above.[5] There were, therefore, two reasons for the reluctance of the public to accept his theory: insufficient published documentation, and the idea that the tumor arose in the hair matrix, which is situated in the bulb and with which the tumors show no direct connection. In his conversation with his pupils, however, he made it clear that he believed that the tumors were of adnexal origin.

In an article written practically at the same time that McCarthy[1] published his textbook (1931), Haythorn[6] reviewed the subject in The American Journal of Cancer, confining his observations entirely to the "basal cell carcinomas." He said: "If we are correct, the neoplasm now regularly called 'basal-cell carcinoma' has less real claim to that name than any of the tumors to which the term was originally applied." He referred, of course, to Krompecher's monograph.[2] As a pupil of Mallory, he approached the subject from the hair matrix angle and adhered to Mallory's opinion that the tumor is derived from hair matrices. Haythorn made a very careful examination of a large number of these carcinomas and his article is excellently documented by photomicrographs illustrating all their varieties. He first considered the matter of apparent downgrowth of plugs of epithelium from the basal epidermal layer and found that continuity between the cells of these plugs and those of the basal layer was only apparent; there was usually a demonstrable, sudden change in the type of the cells where prickle cells were replaced by elements containing the diagnostic longitudinal fibrils of Mallory. He stressed the point that epidermal basal cells are immature prickle cells and demonstrated with photomicrographs more or less rudimentary hair shafts in the cellular nests of the carcinoma. He cited Broders' statement[7] that he had found but one example of this in a series of 252 tumors which he had examined; this he considered to be an understatement.

Haythorn placed much emphasis upon the structure of the membrana propria of the tumor's complexes and investigated it in sections impregnated with silver. He found that it resembled the connective tissue elements of the hair follicle rather than epidermal basement membrane.

Exploring the glandular varieties of the growth, he came to the conclusion that they might be derived from sebaceous glands, which are (after all) merely adnexa of the pilar follicles. He found insufficient evidence of connection with sudoriparous glands to warrant entertaining the assumption that they might be involved in the production of these carcinomas. Nevertheless, on page 1995 of his article,[6] he published two photomicrographs which he said represented a very rare picture; they strongly suggest sudoriparous glandular structure.

In his book on diseases of the breast, Geschickter[8] makes the statement: "The basal-cell cancers of the skin which are adenocystic in type are subepidermal lesions arising from appendages of the skin such as the sudoriferous or salivary glands or the pilosebaceous apparatus." The statement is quite categorical and coincides with the thesis of this paper, excepting that he includes the salivary glands as possible sites of origin of "basal cell carcinoma."

In 1943 Gates, Warren, and Warvi[9] discussed the neoplasms originating in sudoriparous glands; their article is excellent and covers the subject of the nonmalignant varieties very well. It will be noted that they now classify the former "cylindroma" as a solid hydradenoma. This has a striking similarity in its histological architecture to that of a quiescent and very orderly "basal cell carcinoma." It is noteworthy that the two may be closely associated, and hence possibly equally intimately related one to the other. Haythorn[6] cited Owen[10] as having presented the theories of histogenesis of basal cell carcinoma under the name of the authors who have advanced them—

From basal cells: Ewing[11] (and with him the majority of authorities*);

From hair matrices and sebaceous glands: Mallory,[5] Hernaman-Johnson,[12] Paul,[13] Walker[14] (and Haythorn[6]);

From seborrheic patches or senile keratoses: Blasdel.[15]

INVESTIGATION

For many years I have been curious as to which of these theories is the correct one and have been planning to work out some scheme that might serve to elucidate the peculiarities of the "basal cell carcinoma," which will be called "adnexal carcinoma" in the ensuing pages of this text. In the absence of experimental proof, one is forced to fall back upon the interpretation of transitional pictures and the frequently faulty deductions derived therefrom. An experimental approach to the subject is difficult and, at the present writing, is impracticable in our laboratory. The possibility of injecting carcinogenic agents intra-

* Parenthetical additions are by the author.

dermally and intrafollicularly in appropriate animals is self-evident, but carrying out this procedure should include injections into sudoriferous ducts, and most laboratory animals have none. It has, therefore, been decided to attack the problem in the old-fashioned way and to depend upon histological study of representative sections and an interpretation of the findings thus accumulated.

GENERAL REVIEW OF MATERIAL

All examples of adnexal carcinoma available in the collection of the department since 1932 were subjected to a preliminary microscopical survey. There were well over 200 of them and they included every type. After screening out and rejecting the larger tumors as being unpromising of anything new, the sections from the smaller and early growths were selected for intensive examination. The study confirmed the findings of Mallory [4,5] and of Haythorn [6] in almost every particular.

Starting from the assumption that many of the tumors might be of sudoriparous origin, on account of the striking resemblance between adnexal carcinoma and solid hydradenoma and because of the development of the former in an example of the latter recently received, it was immediately evident that far more of them exhibited a definite, if distorted, analogy to pilar units. There was a great variation in the degree of differentiation, some presenting striking similarity to pilar units while others were much further removed from the normal pattern of these, and one was forced to search long for any resemblance. As there appeared to be a group that more closely simulated sudoriparous glands and hydradenomas, these were segregated from those which showed an analogy to pilar or sebaceous units. Pigmented varieties were also sequestered and a few that gave evidence of truly basocellular architecture were likewise set apart.

Description of Types

Several moderately distinctive types were recognizable after a survey of the series and these were arranged, as they are described below, in groups that might indicate their origin rather than afford only morphological differentiation, which means little.

I.   Pilar Type

Roughly three-fourths of the sections represented the pilar type. When this tumor is studied microscopically it is noted that the overlying epidermis is more often atrophic than hypertrophic; the hairs in the region are either deformed or absent, many are crowded to one side and compressed in the adjoining area. Oftentimes they are stretched

out into long, thin structures that skirt the margin of the growth in a parallel arc. The absence of hairs in the skin overlying an adnexal carcinoma might be interpreted as a result of the absorption of hairs into the substance of the growth. (This seems to be true of the Wagner-Meissner corpuscles in the papillae overlying a melanoma; they appear to become incorporated in the neoplasm and to disappear from the derma in its neighborhood.) The pilar type represents several subvarieties depending upon their degree of differentiation, or the amount of degenerative changes that present. They are probably prognostically as well as academically important and are not based solely upon structure.

1. Pilar Type Proper. The pilar type proper imitates the architecture of hair follicles in an unmistakable manner (Fig. 1): There is visible evidence of differentiation into forms that correspond closely with those of normal hair follicles. As one studies the composition of such formations, it is seen that there is a basal layer (possibly manifesting vesiculation) within which there are concentrically arranged islands that imitate various elements of the normal hair follicle. At the core there may be clumps or masses of keratinized material that is much like that of the hair shaft. That Broders found them so seldom is strange; in our sections, stained by the Masson-Goldner method,[16] they took on a bright orange hue and were found in a large number of the tumors examined. These neoplastic complexes may exhibit much of the conventional outline of pilar units, or they may present many sinuous, bulbar outgrowths or processes suggesting pseudopodia. Observation of this well differentiated type of tumor leaves one convinced of its relationship to hairs.

2. Primordial Type. In the primordial type the cells form large spheroidal, conical, or ovoid masses comprising two cellular units: a peripheral delimiting single layer of radially arranged basal cells and a central core of polymorphic elements which are scattered in a haphazard fashion. Such a complex might be compared with a "filled" stone wall; the outer layer of cells is the "face" of matched and fitted masonry, while the core constitutes the "fill" of piled-in, rough stones. The primordial type is very common(Fig. 2) in the earlier forms of the adnexal carcinoma and it may exhibit multicentric origin from the basal layer of the epidermis. It is the prototype of the miscalled "basal cell tumor."

3. Cylindrical-Celled or "Ribbon" Type. The cylindrical-celled or "ribbon" type is rare and consists of rosettes and complex festoons of ribbon-like bands comprising a layer of cylindrical and enlarged cells quite unmixed with any other varieties. Here the "wall" is entirely composed of matched and fitted masonry (Fig. 3).

4. Cystic Type. Rather than a true type, the cystic group constitutes a variant of types 1 and 2 in which there has been vacuolization of the cells with a fusion of the vacuoles that produces cystic cavities; or in which there has been lysis of the stroma with the production of cysts. Both processes may be seen in the stages of development. It is possible that the former, in which the cells become vesicular, represents a change of pilar to sebaceous elements.

II. Sudoriparous Glandular Type

When the sudoriparous glandular type of adnexal carcinoma is present, it is noteworthy that there are few sweat glands in its neighborhood and that those which persist are crowded downward into the derma and subcutaneous tissue. One cannot find bits of hair in these tumors. Instead, there are small onion-like bodies (misinterpreted as epithelial pearls) which are composed of concentrically arranged squamous cells, sometimes containing droplets of keratohyalin and surrounding a tiny lumen that occasionally is lined with a thin layer of eleidin. Such a lumen may expand into a small cyst loosely filled with keratinized scales, and it is then difficult to distinguish from an abortive hair. However, such cysts are frequently noted developing in the terminal, intra-epidermal portion of sudoriferous ducts. The bulk of these growths is composed of complexes of cells arranged in double rows, like imperfect ducts or tubules (Fig. 4). Myo-epithelium may be demonstrated at the margins of the complexes in many instances. Two subtypes are recognized.

1. Adenoid Type. The adenoid type has just been described.

2. Hydradenomatous Type. The hydradenomatous type appears in two forms: (a) A form resembling the solid hydradenoma ("cylindroma"), but manifesting less differentiation and many mitotic figures. Possibly it represents the malignant form of solid hydradenoma (Fig. 5). (b) A type resembling the papillary cystic hydradenoma and, less definitely, the apocrine sudoriparous gland during menstrual stimulation. This is not often seen and it may readily be mistaken for a seborrheic verruca.

III. Basal-Celled Type

As already stated, the basal-celled type is uncommon and, unless it be observed in connection with one or another of the forms just listed, it would be more properly considered to be a plexiform epidermoid carcinoma (Fig. 6). It may occasionally be one, since epidermoid carcinoma frequently develops in the margin of rodent ulcers, the advanced stage of adnexal carcinoma. It is composed of elongated, fusiform cells that grow downward from rete pegs and disrupt them in the process,

later to anastomose with one another in the derma. Here they constitute a plexus of undifferentiated cells which may sometimes exhibit knots, or thickenings of better differentiated elements, and thus acquire more similarity to the pilar type of adnexal carcinoma.

Innervation of the Tumors

Several years ago, while experimenting with the impregnation of neurofibrils with silver, I noticed that the Nonidez modification of the Ramon y Cajal method [17] demonstrated plexuses of very prominent and readily visible nervous filaments in the pilar papillae and about the sebaceous glands. These are described in textbooks of histology. It has been pointed out that they are apparently concentrated in these situations. It is also evident that there are abundant, but finer neurofibrils coursing in the stroma and about the tubules of the eccrine sweat glands. Very few, if any, such fibrils are demonstrable in the papillae of the derma, or in the basal layers of the epidermis.

These facts led to the impregnation of a few small adnexal carcinomas by the above method. The results were so striking that they may constitute the subject matter of a future article. It was found that there are heavy bundles of nonmyelinated fibrils running in the stroma of adnexal carcinomas (Fig. 7). They may arise from the larger trunks of myelinated fibers that abut on the growth. They send a few filaments into the masses of neoplastic cells, where they soon become untraceable; some of them appear to end in terminal buds, or club-shaped extremities similar to nerve endings. While the usual plexuses are found about the normal follicles and sebaceous glands, as well as the sudoriparous glands in the sections, careful examination of the epidermis fails to reveal similar structures.

In the presence of this rich neural plexus in the neoplastic stroma we have further indication of its relationship to hairs, sebaceous glands, and sweat glands, rather than to basal elements of the epidermis in general. The tumor apparently acquires this neural apparatus much the same way as do the adnexal elements of the skin during their embryological development, but this is admittedly pure conjecture.

Pigmentation

Any of the enumerated types of adnexal carcinoma may exhibit more or less pigmentation which may be so intense as to produce a tumor that is grossly identical in its appearance with a malignant melanoma, being quite black and indefinitely outlined. One such tumor developed in a solid hydradenoma of the forehead that had been present for several years in an apparently quiescent condition. The matter of pig-

mentation has been discussed at length by Haythorn.[6] Nine of the 200 or more specimens collected in our department showed more than moderate pigmentation. The melanin is produced by the cells of the tumor and much of it becomes transferred to the cytoplasm of melanophores in the stroma. In dermal sections impregnated with silver, silver-positive melanin is most abundant in the basal layer of the skin, but it is found to extend a considerable distance up the necks of the hair follicles as well.

Arrectores Pilorum

Each hair has its small, smooth-muscular erector; arrectores pilorum may be found scattered throughout adnexal carcinomas. They may have been engulfed by the tumor, but their arrangement hints that they have some relation to the distorted hair follicles that constitute the pilar form of the neoplasm. This is not very strong evidence in support of my theory, but it is at least interesting to note that the small muscles are not much displaced nor destroyed by the tumor.

Investigation of Early Forms of the Tumor in Serial Sections

As it seemed unlikely that anything new might be added to the present conceptions of the histogenesis of this tumor through a morphological study of full-blown examples, two small and very early examples were chosen for investigation. The first was about 5 by 3 mm. in size and came from the nose of a woman in her fifth decade; the second was a very tiny lesion on the aural pinna of an elderly man. A preliminary study of sections indicated the advisability of cutting a series of them and then reconstructing selected areas in the tumor in three dimensions by means of models.

Method of Reconstruction. Accordingly, both tumors were laid down in serial sections and these were studied for suitable foci for tri-dimensional reconstruction. This was carried out satisfactorily and expediently by projecting the sections seriatim upon ordinary cardboard filing cards and outlining in pencil the structures to be built up, numbering the outlines consecutively. A complex was selected in each tumor and projected in the desired magnification. After making the outlines, the complex was measured on the microscopical section with an ocular micrometer and the figures were divided into those taken from the reconstructed model in centimeters. This gave the magnification. As the sections were cut at 6 μ, it was merely necessary to multiply the magnification by 6 in order to find out the requisite thickness of the lamina to be built up from the filing cards, measured in microns. It was found that one card 0.25 mm. in thickness was suitable for a magnification of about $\times$ 40. No attempt was made to adjust the

thickness of the cards with extreme accuracy. If the magnification was × 70 or × 80, two cards were used, the original cut-out being pasted upon another card which was then trimmed to the size and shape of the cut-out. The reproductions were intended to indicate the tridimensional structure in a general way, not to reproduce it exactly. The reasons for this will be clear when the reader has referred to the photographs of the models. The method is quite simple and it obviates the necessity for working with carefully prepared wax sheets. Furthermore, the preparation will not melt if placed in a warm spot. When the outlines were finished, they were cut out with scissors and pasted together in the proper sequence. After the glue had dried they were modelled by painting on molten paraffin and beeswax with a camel's-hair brush until a smooth layer of the mixture had covered the model completely, smoothing out its inevitable inequalities. The model was then scraped with a scalpel after the paraffin coating had congealed and painted in two colors of quick-drying enamel over shellac, the tumor in vermilion and the rest of the model in light blue.

Study of Models. Study was not limited to the finished models, for as each section was glued into place the diagrammatic outlines on its surface were observed and the manner of growth of the tumor became much more obvious than any bidimensional sections could render it. Comparison of doubtful areas in the model with the microscopical sections from which they were drawn enabled the correction of any misconception. Several points became manifest. The growth appears to begin as a loosening of elements of the rete in the neighborhood of the debouchment of the hair sheaths and sweat ducts through the epidermis. Thus cells accumulate until small nodules are produced, but these lie just *outside* or above the basal layer of cells and comprise a welter of polymorphic elements that exhibit numerous mitotic figures. These complexes appear to push the basal layer downward, bowing it out into the dermal connective-tissue (Fig. 8). In this way, conical or bulbous mammillary processes are formed in the derma without producing any corresponding distortion on the surface of the epidermis. Larger examples of these processes tend to mushroom out below the epidermis and gradually to take on bizarre forms not unlike those of certain tubers. They are usually covered by smaller nodose or bulbar processes that stud their surfaces and remind one of the sebaceous buds along the shaft of a pilar primordium, or even of small hair follicles. The large tuberous complexes of the tumor are attached to the epidermis by a relatively small and short stalk which represents the original conical or bulbous beginning of the growth. Such a stalk supports the complex shown in reconstruction 1 (Figs. 9 and 10) and is shown in microscopi-

cal sagittal section in Figure 18. The greatest over-all longitudinal diameter of this growth was about 2,465 μ; its width, 820 μ; and its thickness as reproduced, 570 μ. There was more of the tumor in sections that were cut and discarded before the serial sections were prepared, so that it was really somewhat thicker than this. The flattened pyramidal pedicle that supports the complex has a diameter of 799 μ at its widest point, but it is only 210 μ in thickness. The pedicle arises from the epithelium in the immediate vicinity of the neck of the pilar unit shown in the model. It will be noted that the section portrayed in Figure 18 shows the complex to be of the primordial type that exhibits considerable differentiation toward the pilar variety. Variations in the size and shape of the neoplastic complexes are observed; sometimes they are merely small conical projections which have not as yet grown into the larger variety; sometimes there are small areas of anastomotic columns of basal cells, but these are few in the early forms of the growth.

Interpretation of Reconstructed Tumor. Interpretation of these phenomena is admittedly hazardous. The cells that form the initial masses between the basal layer and the rete might be produced by the multiplication and emigration of basal elements, or by the multiplication of the pleomorphic central cells *in situ*. The latter possibility seems to be the case, as can easily be determined by noting the much larger number of mitotic figures among those cells as compared with the elements of the basal layer. They abut on prickle cells on the one hand and basal cells on the other, merging imperceptibly with either type along the border of the mass. A few prickle cells may lie among them; as for the basal cells, they appear to be forced downward by the accumulation of pleomorphic cells until they are pushed into the depths of the derma. They usually maintain their continuity with the basal layer of the epidermis, constituting the envelope of cells already described. This accounts for their radial orientation in respect to the tumor complex. Examined under high magnification, the cells of the central mass have little to distinguish them from any other primitive pleomorphic cell until they undergo differentiation. When this occurs, they mimic elements of the hair follicle, sebaceous, or sweat gland and do not produce prickle or squamous cells that typify the epidermis.

The cellular structure of the large, tuberous complexes is too familiar to delay us here; it has been described by many writers since Krompecher's time. Their observations as to occasional differentiation of a varying degree, the production of abortive or misshapen hair shafts, and the formation of cysts are all readily confirmed. One point has been overlooked, or underemphasized: the tendency of some of the

neoplastic complexes to send forth occasional tubular processes reminiscent in their shape of the ovipositors of certain fish or insects. Examination of these abortive tubules, which project only 1 mm. or so into the derma and then end abruptly in a tapering tip, shows that they bear an extraordinary histological likeness to sudoriferous ducts. Such a structure is shown in reconstruction 2 (Figs. 11 and 12), much enlarged and dominating the model. A microscopical section through it is illustrated in Figure 13. Another smaller abortive tubule is partly hidden behind the large hair follicle in reconstruction 1. Sometimes an even more abortive beginning of such ducts may be noted: a group of cells sprouts an elongated mammillary process from the surface of one of the larger tumor-complexes, forms a short prong that appears in three or four serial sections, and is then lost. On reconstruction it amounts to little more than a tiny and somewhat fusiform spike, or elongated "tear drop."

DISCUSSION

Hypotheses

An attempt to explain the histogenesis of adnexal carcinoma on the basis of the data thus accumulated will, of necessity, be somewhat didactic. Let us consider what might be expected of the neoplastic elements were they primarily epidermal basal cells (as has for so long been claimed) and were they subjected to carcinogenic stimuli: what would a basal cell do under such circumstances? Normally it differentiates into prickle cells, next granular elements, and finally into keratinized squamous cells. Should it pursue this line in a tumor, it would pile up keratinized scales on the surface and produce a verruca; should differentiation stop in the prickle cell phase, the result would be acanthosis and a condyloma; should it fail to differentiate at all and remain of the basal cell type, it would divide and multiply to form a totally undifferentiated, plexiform tumor, the plexiform epidermoid carcinoma. It would not produce dermal adnexa, however, unless it was a certain specialized basal cell that was destined to do so. This will be discussed later.

Could basal cells form anything like this tumor, the adnexal carcinoma, and if so, where and how? In the embryo there are certain areas where basal cells become specialized to produce epidermal adnexa: hair, sebaceous and sudoriparous glands. During the process of doing so they form primordia that are strikingly similar to the structures that typify adnexal carcinoma (Figs. 14, 15, and 16).

Histogenesis and Histology of the Hair

The embryonal primordium of the hair is merely a downgrowth of two types of cell from the epidermis: an outer layer of radially orientated cylindrical elements and a core of polymorphic cells of an indifferent type, the *embryonal* hair matrix. From the sides of the plug thus formed small lateral buds are given off; they may develop into sebaceous glands, or they may be resorbed by rearrangement of their elements and thus disappear (Figs. 14 and 15). The hair that results from the differentiation of this simple primordium is, in effect, simply a column of invaginated epidermis. The keratinized layer is molded into a hair shaft, the granular layer persists for some distance up the sheath, the malpighian layer becomes altered to form the cells of Huxley and Henle, while the basal layer undergoes vesiculation until most of its cells are converted into cylindrical sacs. In the base of the bulb is a small group of undifferentiated cells which renew the shaft and constitute the *adult* hair matrix. Around the cellular sheath is a prominent reticular membrane with its cells lying parallel with the contour of the bulb and shaft; this begins near the opening of the sheath, surrounds it and the bulb, but is not comparable with the epidermal basement membrane which is more delicate, more reticular, and less compact. At the tip of the bulb there is an indentation into which a papilla of connective tissue and neurofibrils is received. No attempt is made here to detail the structure of the hair further, but it should be pointed out that among the 200-odd tumors examined, some analogy to the above outline was found in the majority of them. Abortive hair shafts, small collections of cells with keratohyaline granules, vesiculation of the basal cells surrounding the tumorous masses, and rudimentary papillae of connective tissue and nerves projecting into indentations in these masses; all of these were noted. The fact that the more primitive primordium was frequently imitated has been sufficiently indicated.

It seems that this similarity is not fortuitous, but that the tumor is merely recapitulating, in its neoplastic and disorderly way, the embryological process that precedes the formation of the dermal adnexa; in some instances it gets no farther than the embryonal phase, while in others it nearly succeeds in producing hairs. That the tumor arises in the *embryonal* rather than in the *adult* hair matrix is a more satisfactory explanation than Mallory's, as the former is situated exactly where these tumors appear to originate, rather than near the hair matrix of the bulb with which I could observe no direct connections. It is unfortunate that there is no definite explanation as to why embryonal primordia should persist in adult epidermis. Lacking direct evidence,

we must fall back upon one of two hypotheses: Virchow's ancient theory of cellular rests, or a retrogressive change in certain basal cells near the hair follicles, whereby they are returned to an embryonal or proliferative state.

Histogenesis and Histology of Sebaceous Glands

While examining the more glandular types of adnexal carcinoma one will occasionally be found which exhibits vacuolization of its cells to a degree that indicates sebaceous origin. Bearing in mind the fact that the tumors often arise at a point where the sebaceous ducts empty onto the surface, it would not be strange if these were included in the mechanism of the histogenesis of adnexal carcinoma. Sebaceous glands may be dismissed with the remark that they develop from the pilar primordia, as already indicated. Their ducts are connected with lobules of large cells containing lipid material that is discharged by the holocrine disintegration of the cells. These glands are usually very numerous in the neighborhood of adnexal tumors, possibly because of the fact that the latter most usually develop on the nose or upon parts of the face where there is much sebaceous secretion.

Histogenesis and Histology of Eccrine Sudoriparous Glands

It will be unnecessary to go into the finer differences between eccrine and apocrine glands, the former being much more pertinent to the subject under discussion. An eccrine sudoriparous gland is a very simple affair that develops by a downward growth of a conical primordium from the base of the epidermis. This type of primordium is common to the hairs, sweat glands, and the mammary glands. In the case of the sudoriparous variety, it tends to mushroom out under the epidermis as it grows, and from its apex a slender stalk of cells works its way downward into the corium or subcutaneous tissue and coils itself into a globose knot. The larger mass of primordial cells persists for a time and then disappears (Fig. 16). The epithelium of the sweat glands is simple, cuboidal and unspectacular. In the proximal, secreting portion of the tube there are two layers of cells, an inner cuboidal layer and an outer stratum of strap-like elements, the "myo-epithelium." The secretory tube becomes more and more compact as it approaches the epidermis and its cells begin to show granules of keratohyalin. Finally, the duct is a simple spiral channel in the keratinized layer of the epidermis, its lumen lined with a layer of eleidin. In the region of the coil, neurofibrillae are numerous and surround the tubules.

In those tumors which might be attributed to such glands it is possible to find small concentrically whorled structures with a tiny lumen

also lined with eleidin; it is well to note that a cross section of a sudoriferous duct as it joins the epidermis is almost identical in its appearance with these small structures. The production in the tumor of teat-like processes resembling sudoriferous ducts has also a conspicuous bearing on this argument, as well as the presence in many of the growths of a peripheral layer of myo-epithelium about the neoplastic complexes. To these points should be added the presence of a rich nervous supply in the stroma of the tumor and of sweat glands alike.

Metastases

It is well known that these tumors seldom metastasize and only then when they are large and extremely ulcerated; only one in our collection has done so. This was a large subaural rodent ulcer. A study of the metastases in lymph nodes is of value as it represents the trend of development in the tumor when this has been completely removed from its natural environment. It is found that the metastasis adheres closely to the basic pattern of the primary growth; it forms the same type of complexes and, in the instance cited, took on the pilar type of the parent tumor (Fig. 17). Were such a growth composed of undifferentiated basal cells of the epidermis, the metastasis should present nothing more than a plexiform arrangement of fusiform epithelial cells.

RECAPITULATION

As may be seen from the preceding pages, correlation of the various types of adnexal carcinoma and certain phases of the life history of the dermal adnexa is not a difficult matter. The primordial type of this tumor reproduces distorted pictures of the embryological development of the hairs, sebaceous and sudoriparous glands. Whether every adnexal carcinoma begins as a focal multiplication of primordial cells in the region of the mouths of the hair follicles, or whether some of them may grow directly from distorted adnexal elements is hard to determine. It is also probable that the adnexal carcinoma may develop from more than one type of primordium, as one tumor may show areas that indicate pilar origin and other areas that point towards a sudoriparous genesis. It is true, however, that in all of the very early examples of the tumor examined in this series, the formation of distorted primordia was the outstanding feature.

That these tumors do not arise from basal cells as such is indicated by the fact that they fail to differentiate along epidermoid lines. Their differentiation is entirely in the direction of pilar, sebaceous, or sudoriparous structure. It is the chief purpose of this article to divert attention from the epidermal basal cells toward those specialized basal cells

which form adnexal primordia. Secondarily, it is desirable to change the emphasis on the hair matrix to one on the adnexal primordium. In a general sense these tumors are derived from basal cells, as are all epidermal tumors, but this is no reason for calling them "basal cell carcinoma"; an epidermoid carcinoma would have just as much claim to that name.

The currently widely maintained concept that the adnexal carcinomas are composed of undifferentiated basal cells is quite incomprehensible to me; even a cursory examination of sections from one of them will prove that there is much differentiation going on in most of the neoplastic areas. In some tumors it is merely toward the embryonal primordium, which is the least specialized in its architecture; in others the trend is toward something reminiscent of dermal adnexa; while in a small group the differentiation falls just short of the production of such adnexa.

CONCLUSIONS

A study of the structure of early examples of "basal cell carcinoma" indicates that this neoplasm originates in distorted primordia of dermal adnexa rather than from ordinary epidermal basal cells. The tumor imitates the embryonal development of hairs, sebaceous and sudoriparous glands. The reasons for these assumptions are as follows:

1. A recapitulation of the histogenesis of dermal adnexa is present in the majority of the tumors.

2. There is a distinct similarity in the architecture of the growths to that of these adnexa in their adult state:

    (a) Abortive hairs are formed.

    (b) Abortive sudoriferous ducts may be observed in some instances.

    (c) There is a copious and complex development of neurofibrillae in the stroma of the tumors, comparable only to that seen in adult, normal adnexa.

3. The indications are that a given adnexal tumor may take origin from any or all three types of adnexal primordia.

4. Metastases in lymph nodes undergo the same type of differentiation and form the same abortive adnexal structures as are found in and produced by the primary tumor from which they have come.

REFERENCES

1. McCarthy, L. Histopathology of Skin Diseases. C. V. Mosby Co., St. Louis, 1931.
2. Krompecher, E. Der drüsenartige Oberflächenepithelkrebs. Carcinoma epitheliale adenoides. *Beitr. z. path. Anat. u. z. allg. Path.*, 1900, 28, 1–41.

3. Krompecher, E. Der Basalzellenkrebs. G. Fisher, Jena, 1903.

4. Mallory, F. B. Recent progress in the microscopic anatomy and differentiation of cancer. *J. A. M. A.*, 1910, 55, 1513–1516.

5. Mallory, F. B. Principles of Pathologic Histology. W. B. Saunders Co., Philadelphia, 1914, p. 371.

6. Haythorn, S. R. Studies on the histogenesis of the so-called "basal-cell carcinoma." *Am. J. Cancer*, 1931, 15, 1969–2000.

7. Broders, A. C. Basal-cell epithelioma. *J. A. M. A.*, 1919, 72, 856–860.

8. Geschickter, C. F. Diseases of the Breast. J. B. Lippincott Co., Philadelphia, 1945, ed. 2, p. 604.

9. Gates, O., Warren, S., and Warvi, W. N. Tumors of sweat glands. *Am. J. Path.*, 1943, 19, 591–631.

10. Owen, M. Basal cell carcinoma; a study of 836 cases. *Arch. Path.*, 1930, 10, 386–394.

11. Ewing, J. Neoplastic Diseases. W. B. Saunders Co., Philadelphia, 1928, ed. 3, pp. 877–884.

12. Hernaman-Johnson, F. The treatment of rodent ulcer (basal cell epithelioma), with especial reference to recurrence. *Lancet*, 1926, 1, 389–391.

13. Paul, N. Observations on the origin, causation and treatment of rodent ulcer. *M. J. Australia*, 1923, 1, 85–90.

14. Walker, N. The pathology of rodent ulcer. *Tr. Path. Soc. London*, 1894, 45, 172–174.

15. Blasdel, H. E. Basal cell carcinoma. *J. Kansas M. Soc.*, 1923, 23, 338–341.

16. Goldner, J. A modification of the Masson trichrome technique for routine laboratory purposes. *Am. J. Path.*, 1938, 14, 237–243.

17. Foot, N. C. Useful methods for the routine examination of brain tumors. *Am. J. Path.*, 1938, 14, 245–252.

--- ---------

[Illustrations follow]

DESCRIPTION OF PLATES

PLATE I

FIG. 1. True pilar type of adnexal carcinoma. Of note is the general resemblance of the central complex of cells to an embryonal pilar primordium. For comparison with Figure 15. Concentrically arranged cells at the center of the field simulate those of the neck of a hair sheath. × 90.

FIG. 2. Primordial type of adnexal carcinoma, its simplest form. For comparison with the diagrams in Figure 14. × 90.

FIG. 3. Cylindrical-celled or "ribbon" type, which does not betray its origin beyond presenting a slightly glandular appearance. × 90.

FIG. 4. Sudoriparous glandular type of the neoplasm. A long duct runs diagonally across the field; it may be a normal structure involved in the tumor or it may be of neoplastic origin. × 90.

Foot

Adnexal Carcinoma of the Skin
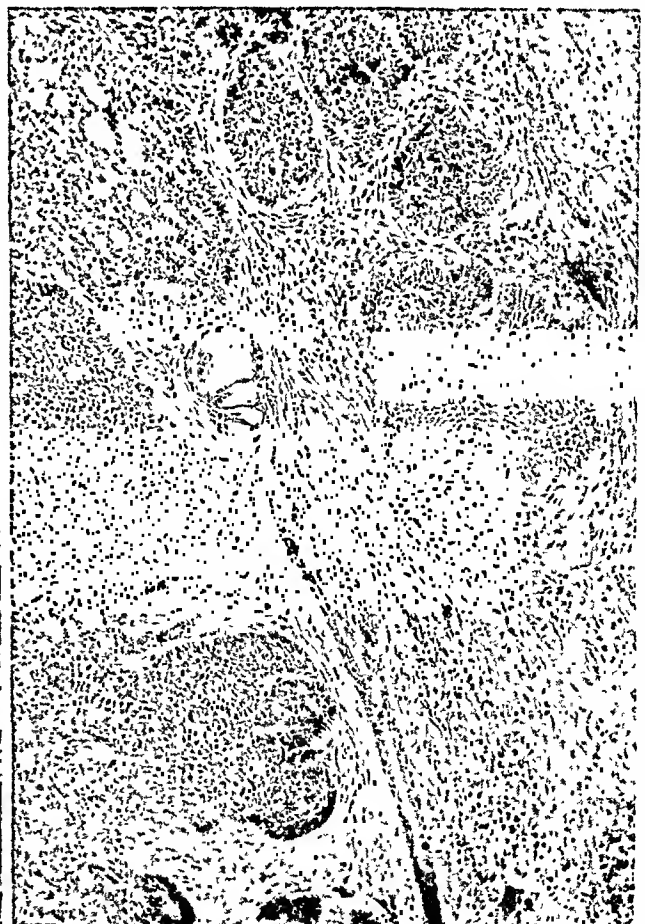
PLATE 2

FIG. 5. A typical solid hydradenoma for comparison. There are several ducts comprising two layers of cells, a particularly well developed one being apparent at the left of the field. × 80.

FIG. 6. Basal-celled type. There is some question whether this is not, in reality, a plexiform epidermoid carcinoma. × 80.

FIG. 7. Adnexal carcinoma impregnated with silver by the Nonidez-Ramon y Cajal method. All black filaments represent neurofibrillae. This technic does not impregnate reticulum to any extent. Of note are the small buds or bulbous terminals in the basement membrane of the complex at the upper right portion of the field. × 460.

FIG. 8. The earliest changes in the skin of the aural pinna in adnexal carcinoma. For comparison of the angular downgrowth with the diagrammatic primordium shown in Figure 16. × 60.
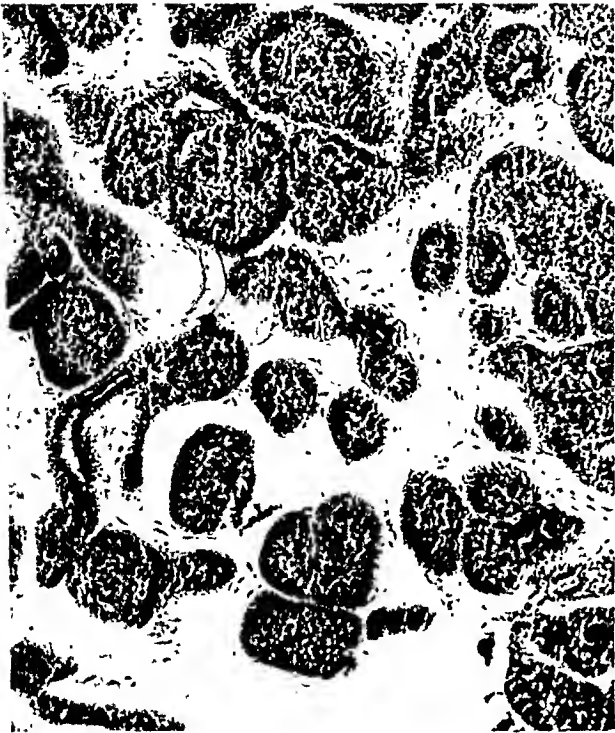
5
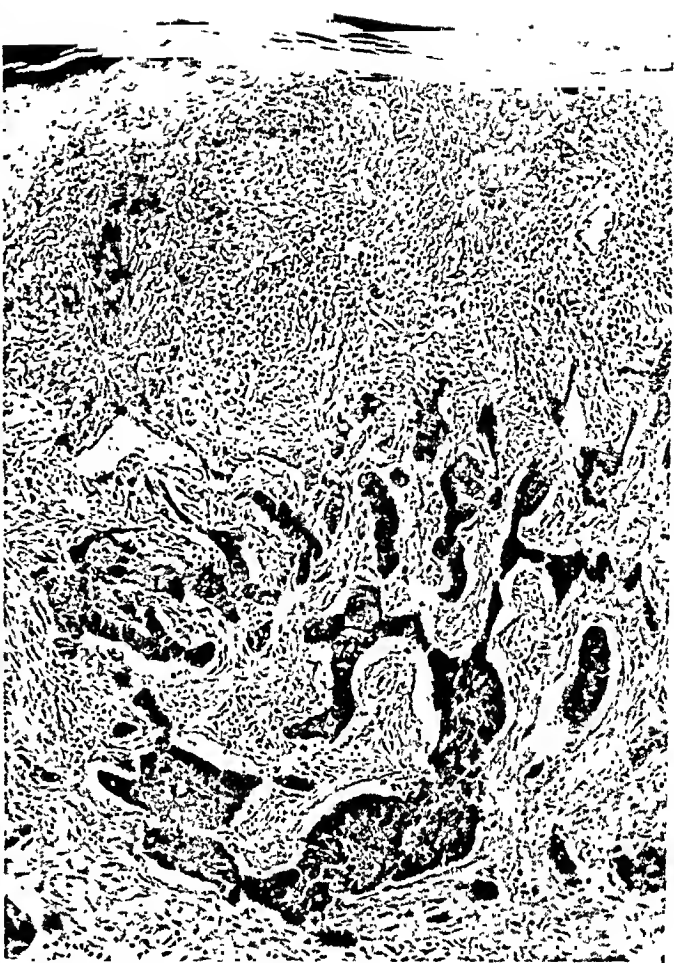
6
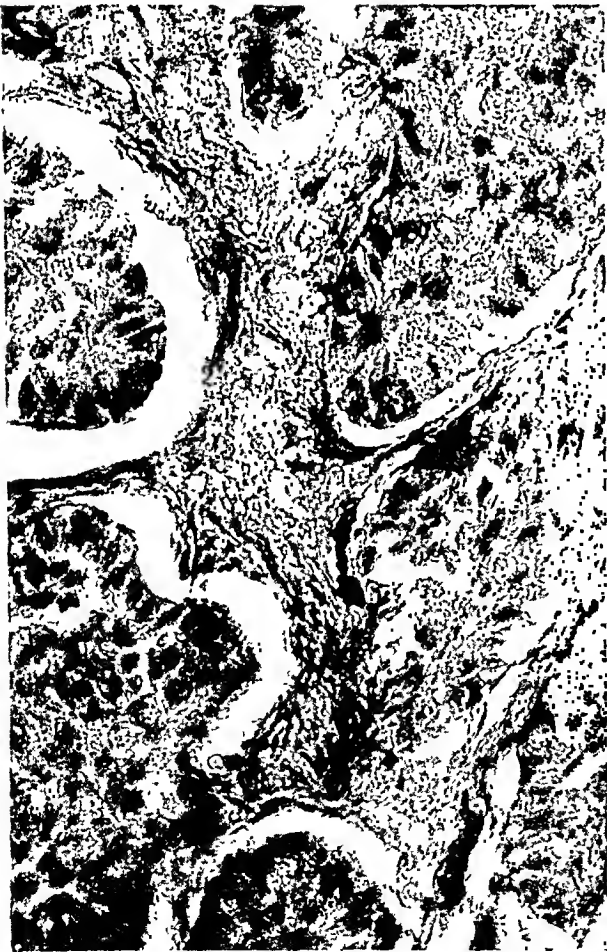
7
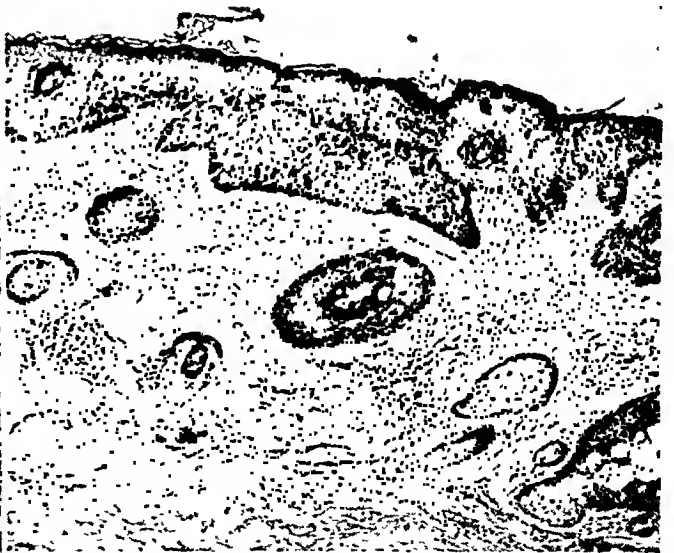
8

PLATE 3

FIG. 9. Reconstruction 1. Enlarged reproduction of a tuberous complex of adnexal carcinoma (gray) arising from a pedicle near the opening of a hair sheath (white, at top). Of note are the incipient sweat duct projecting near the hair sheath and the lobulated contours of the neoplastic mass. Model. $\times$ 70; photograph, about $\times$ 50.

FIG. 10. Reverse aspect of reconstruction 1. Two lobes of the tumor (gray) surround the neck of the hair sheath (white). The pedicle of the bilobed complex arises just behind the hollow channel of the hair sheath. About $\times$ 50 as photographed.

FIG. 11. Reconstruction 2 resembles the primordium of a sudoriparous gland. An abortive sudoriferous duct is seen projecting (in gray) above the hair sheath (white) at the right. $\times$ 70.

FIG. 12. Reverse side of reconstruction 2. All of the grayish portion is neoplastic tissue, the skin and a sebaceous gland are white. $\times$ 70.

Foot

Adnexal Carcinoma of the Skin

PLATE 4

FIG. 13. Sagittal microscopical section of the complex in reconstruction 2. Of note is a sudoriferous duct running down from the primordium-like mass. For comparison with Figure 16. × 95.

FIG. 14. Two early primordia of hairs from a 3-months-old embryo. No. 1 is the earliest beginning, no. 2 is slightly more advanced. "HK" is the embryonal hair matrix. (Fig. 304 of Maximow and Bloom, Textbook of Histology, 1931, ed. 1. Reproduced by permission of W. B. Saunders Company and Dr. William Bloom.)

FIG. 15. Two pilar primordia in more advanced stage of development. Of note are the accessory primordia at the necks of these embryonic follicles. For comparison with Figure 1. (Figure 305 of Maximow and Bloom, Textbook of Histology, 1931, ed. 1. Reproduced by permission of W. B. Saunders Company and Dr. William Bloom.)

FIG. 16. An eccrine sweat gland from the volar surface of the index finger. For comparison with reconstruction 2, Figures 11 and 12. (Figure 299 of Maximow and Bloom, Textbook of Histology, 1931, ed 1. Reproduced by permission of W. B. Saunders Company and Dr. William Bloom.)

13

14

15

Foot
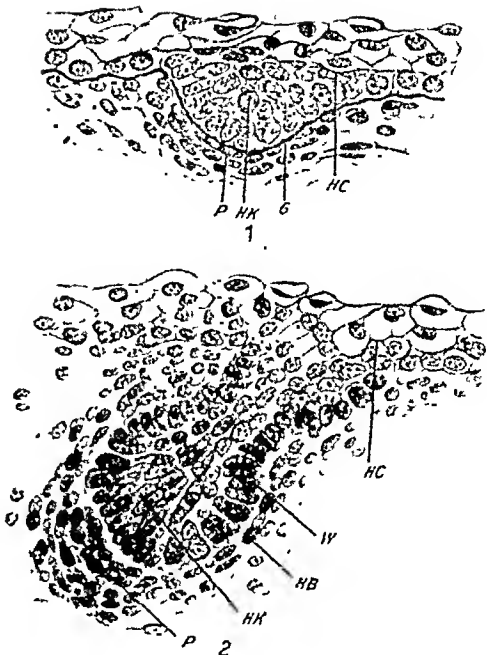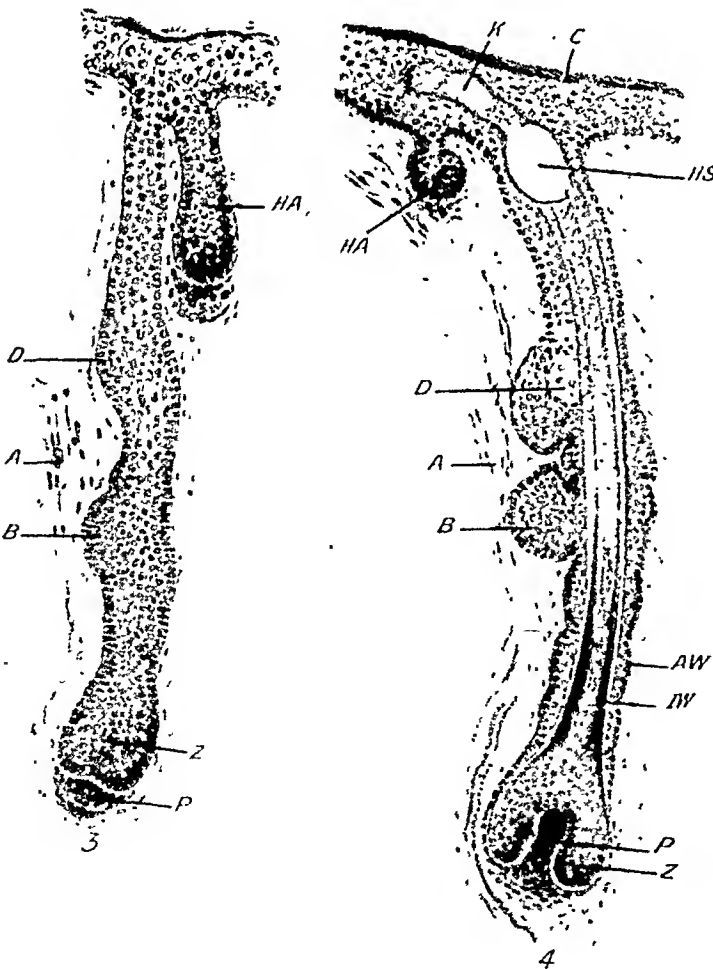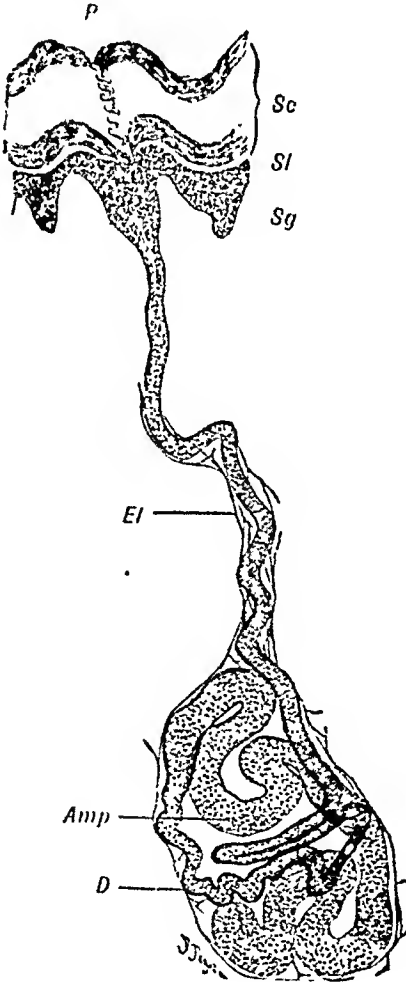
16

Adnexal Carcinoma of the Skin

PLATE 5

Fig. 17. A field from a metastasis of adnexal carcinoma in a lymph node in the subaural region. In it, differentiation toward pilar or sudoriparous structure is maintained in spite of the removal of the growth from its connection with skin. Some of the concentric structures contain keratinized débris; this might be abortive hair shaft material or keratinized lining of sudoriferous ducts.

Fig. 18. A sagittal section of the neoplastic complex in reconstruction 1 (one of a series from which it was built up). Of note is the very tenuous pedicle arising from the epidermis. The primordial type predominates. × 80.
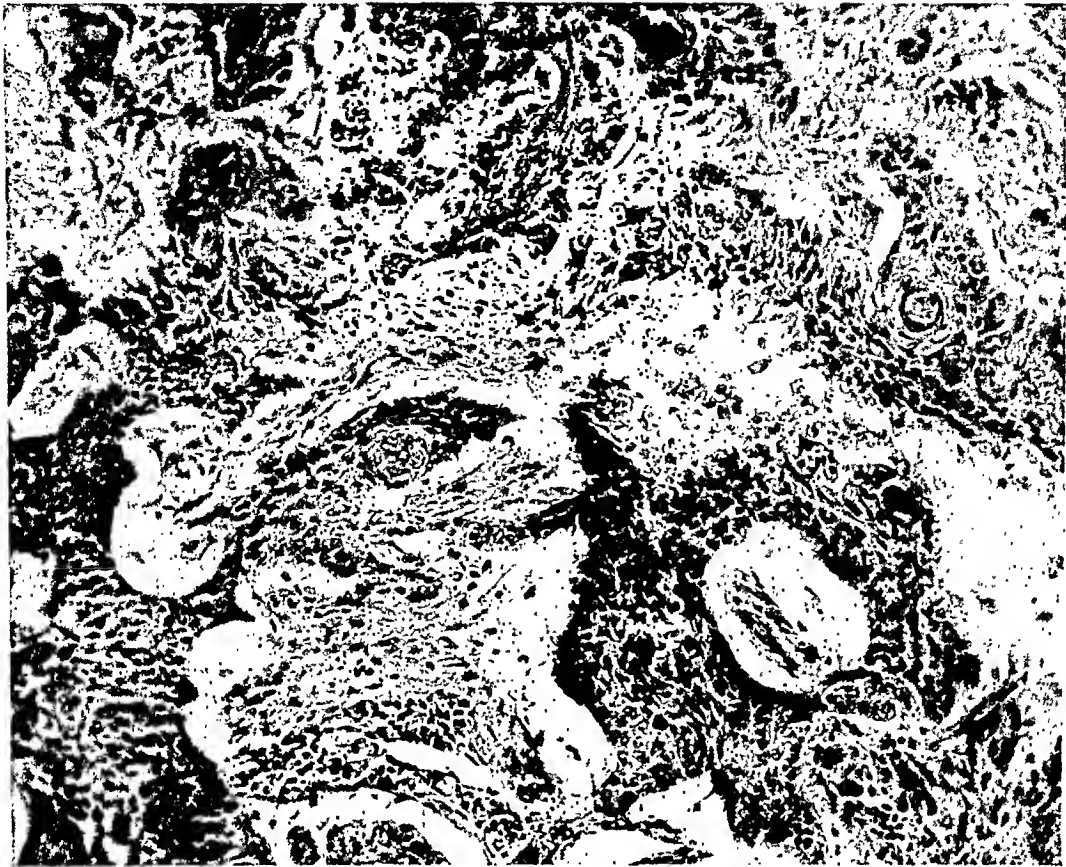
17

18

MUMMIFIED EPIDERMAL CYSTS

(So-Called "Calcified Epitheliomas")*

Major Lester S. King, M.C., A.U.S.†

(*From the Laboratory of the William Beaumont
General Hospital, El Paso, Texas*)

The tumor known in the literature as "calcified epithelioma" is relatively infrequent. In 1933 Ch'in[1] reported 10 cases in a group of 22,000 consecutive surgical specimens, while in 1944 Highman and Ogden[2] described 12 instances from more than 24,000 specimens. Côté[3] discussed 12 examples in 1936, observed over a period of 8 years at Leeds General Infirmary. Fevre, Huguenin, and Paiz[4] found 9 cases in a period of 9 years at the Institut du Cancer in Paris, and discussed them in 1938. Sutton and Sutton[5] reported a single case, while other case reports have been described in the American and foreign literature[6-8] including multiple examples in a single patient. Gromiko[9] discussed a malignant transformation of one of these tumors. Warvi and Gates,[10] in their analysis of 566 cystic tumors of the skin, briefly mentioned 11 as calcified and one as ossified, but did not make it clear whether these conformed to the published reports of calcified epithelioma, or whether they represented simple calcification of epidermal cysts.

The nature of the tumor has provoked considerable difference of opinion. Most authors relate it to epidermoid cysts, "atheromas" of the skin, sebaceous cysts, and similar categories. It has also been considered a lesion *sui generis* as well as related to basal cell carcinomas. Warvi and Gates,[10] in their brief but penetrating discussion, enumerated different classes of tumors which may calcify, and which they subsumed under the generic term of calcified epithelioma.

It seems inescapable that more than one type of tumor has been so designated. Calcification may or may not occur. Cells may be squamous or basal in type. Viable epithelium may or may not be present. Most examples are clearly benign, but malignancy has been reported as well as benign recurrences. It is difficult to find any single characteristic which applies to all reported examples.

In my own material tumors of two distinct types have been encountered, each of which corresponds to some of the reported cases. It is the purpose of this communication to analyze these two types and trace their histogenesis.

My material is drawn from 7,500 consecutive surgical specimens.

Nine cases have been selected, divisible into two categories. The first group, of 8 cases, includes 2 transitional examples. The ninth case is clearly different. All epidermal cysts in the files, to the number of 123, were also reviewed. The two transitional cases had been originally classified as epidermal cysts.

The patients, 8 men and one woman, ranged from 11 to 63 years of age. The tumors were located on scalp, face, neck, back, arm, and thigh. In duration the extremes were 3 months (of questionable accuracy), to 10 years. The gross pathologic features were not characteristic. The specimens varied in size from 0.8 to 3.5 cm. in greatest dimension. They were roughly and approximately encapsulated, with consistency varying from rubbery to hard. All specimens could be incised, but several required decalcification before sectioning. The exposed cut surfaces varied from grayish white to mottled tan, and from smooth to granular and crumbly. Small cystic spaces, 1 to 2 mm. across, were seen in 3 instances. For purposes of analysis, brief individual microscopic descriptions are given.

MICROSCOPIC DESCRIPTIONS

Case 1. In the lower corium was a well circumscribed, encapsulated tumor consisting of small masses of epithelial nature, separated by bundles of collagen. The epithelial masses were entirely nonviable, and were composed of ghost-like shadows and outlines. They appeared to have been originally squamous in character. Distinct cytoplasmic outlines were visible, but no intercellular bridges were seen. Most cells showed a faint nuclear shadow. There were extensive deposits of deep-blue-staining material, identifiable as calcium, which encrusted the nuclei and was present as a fine dusting within the cytoplasm. The stroma intervening between the cell masses was very poorly vascularized. A few multinucleated foreign body giant cells with very well stained nuclei were seen, in contrast to the mummified epithelium. No bone formation was present. Figure 1 illustrates this tumor. The mummified epithelial masses with distinct cell outlines show heavy deposition of calcium granules around the periphery, with a sparser dusting in the central portions. Shadowy outlines of epithelial nuclei can still be seen. The poorly cellular and poorly vascularized stroma is composed of dense collagen.

Case 2 was similar to case 1 in all essential respects.

Case 3. The tumor of case 3, generically similar to the preceding, showed a few noteworthy differences. The masses of mummified epithelium, with distinct cell boundaries, were much larger, and there was a corresponding diminution in the amount of fibrous stroma. Abundant

calcification was present, proved by the von Kossa stain. But in addition, extensive amounts of iron-containing pigment were observed, confirmed by the Prussian blue reaction. This pigment was seen as a fine dusting of epithelial cytoplasm and nuclei, and in large masses within the stroma. Iron and calcium co-existed in the same cell groups.

Case 4 was of unusual interest, since the pathogenesis of the tumor could be clearly traced. The patient was a soldier, 38 years old, who had suffered multiple battle wounds. One shrapnel fragment struck him in the forehead. The wound drained for about 2 months and then a tumor developed over the next 6 months. Histologically, the picture was very similar to that of cases 1 and 2, except that there was a vigorous round-celled infiltration in the stroma, with large numbers of foreign body giant cells. Calcification was much less intense. Figure 10 illustrates this case. In one portion of the tumor there was a small, typical epidermal cyst with a narrow peripheral rim of viable squamous epithelium, and a content of partly calcified, fused, keratinized material, in which cell outlines were not visible.

Case 5. The tissue of case 5 consisted of relatively large masses of mummified epithelium, showing variable amounts of calcification encrusting both nuclei and cytoplasm. The squamous character of the epithelium was clearly visible, but the staining reactions did not indicate viability. In parts of the specimen the mummified cell masses were closely invested by well formed bony trabeculae with clearly staining bone corpuscles and many scattered cement lines. There was no osteoblastic activity. Elsewhere there was the usual fibrous stroma. Figure 2 illustrates the excellently formed bony trabeculae, with marrow spaces. The bone is in immediate apposition to the nonviable epithelium, the cell boundaries of which are clearly visible.

Case 6. Pathologically, case 6 was virtually identical with case 5.

It is clear that these 6 cases are all essentially similar. The presence or absence of bone formation in the stroma is of no fundamental significance. It must be interpreted as a metaplasia of the stroma, present in some instances, absent in others, without affecting the fundamental nature of the tumor. Similarly, the very large amount of iron in addition to the calcium in case 3 may be considered an indication of unusual local metabolic factors, in a case otherwise similar.

The traumatic origin of case 4 distinguishes it from the others in this series which arose spontaneously. That epidermal cysts or implants may arise from local trauma, often extremely slight, is universally recognized. That a typical example of "calcified epithelioma" should arise from definite trauma strongly suggests the kinship of this lesion with the epidermal cyst.

Discussion of Simple Epidermal Cysts

Before considering the transitional cases, simple epidermal cysts may be considered. In a series of such cysts certain definite gradations can be observed. As the basic type may be designated the familiar form in which there is a narrow intact lining of squamous epithelium with well defined basal and prickle layers and a definite, although narrow, kerato-hyaline layer. The cyst contents consist of flaky, loosely lamellated keratotic material (Fig. 5). Sometimes this material is inspissated, fused, and partly hyalinized. In routine stains the flaky squames are tinged a faint blue or lavender, while the dense material is pink. Occasionally, faint traces of nuclei are seen in the cyst contents, but cytoplasmic outlines and membranes are lost.

The lining epithelium is not always regular and even. Occasional areas of proliferation are observed, in which conical, bud-like, papillary, or trabecular processes extend inward (Figs. 6 and 7). The epithelium, instead of being entirely orderly and reproducing the structure of the normal epidermis, may exhibit dyskeratosis. Instead of a definite, flattened, keratohyaline layer, the marginal cells may be enlarged, rounded, and pale-staining, with frequent perinuclear vacuoles, and alterations of the size and staining qualities of the nuclei. Such pale, ballooned cells are seen in Figures 6 and 9. The cyst contents may then reveal certain differences from the more usual type. Instead of consisting of loose or dense, simple, keratinized material, the contents may exhibit parakeratosis (Fig. 6) with persistence of nuclei which are flattened and pyknotic. There is also a tendency towards persistence of cell outlines and cell membranes. Epithelial whorls, of shadowy character, may be present in the center of cysts, surrounded by inspissated, fused, keratinized material. In these whorls cell outlines are clearly preserved, and the plump cell contours contrast with the surrounding, fused keratin.

These variations in cyst contents, as compared with Figure 5, are apparently related to the character of the lining epithelium. Whorls, persisting nuclei, and preservation of cell contours and membranes are seen where the lining epithelium shows disorder of architecture, alterations of cell structure, and departure from the normal epidermal pattern. To such changes the term dyskeratosis * may be applied, relative to the viable epithelium forming the rim of an epidermal cyst.

The contents of epidermal cysts are prone to calcification. I have

---

* This use of the term differs from that employed by Highman and Ogden [2] in their discussion of "calcified epithelioma." They apparently applied it to nonviable degenerated cells of the type that I have called "mummified cell masses." In my opinion the term should apply only to viable epithelium showing disorders of cellular structure and organization. There is no necessary implication of carcinoma.

never observed calcification when the contained material is of the loose lamellated type (Fig. 5), but only when it is dense and inspissated. Calcification of the contents is independent of the degree of regularity or of dyskeratosis of the lining epithelium.

Another very frequent complication is rupture and destruction of the epithelial rim, with attendant granulomatous reaction. Leukocytes and foamy macrophages vary in number. In one instance actual bone formation was observed in the granulation tissue bordering such a cyst. The epithelial lining in this instance was completely destroyed, but occasionally persisting partial arcs of epithelium lay adjacent to granulomatous or fibrous areas. .

These variations in structure of .epidermal cysts are of importance in considering the histogenesis of so-called calcified epithelioma. .

TRANSITIONAL CASES

Case 7. Around the greater part of the periphery of the cyst in case 7 there was a delicate fibrous capsule, within which was a layer of squamous epithelium showing a few minute papillary buds. At the inner free margin the epithelial cells were large, pale, granular, and obviously degenerating (Fig. 9). Many of these nuclei were shrunken or pale-staining. The central portion of the mass was composed largely of keratinized material, staining dull red, in which cytoplasmic outlines were excellently preserved (Fig. 8). Nuclear outlines were ghostly and shadowy. Numerous large masses of calcium salts were present. At one edge the epithelial lining was incomplete and there was invasion by granulation tissue containing many large macrophages, a few of which were lipid-bearing. This granulation tissue, affecting only a small part of the specimen, penetrated among the masses of keratotic material, breaking it up into smaller irregular masses. Polymorphonuclear leukocytes were conspicuously absent.

Case 8. The specimen from case 8 was a small cyst from the upper eyelid, present for 3 years. It was essentially similar to that of case 7 except for its smaller size. It presented in excellent fashion the incomplete layer of lining squamous epithelium which, away from the basal layer, revealed marked dyskeratosis. The center of the cyst was filled with masses of mummified material similar to those shown in Figure 10. Although the lining epithelium was not complete, there was no significant invasion of the cyst content by connective tissue.

The specimens from cases 7 and 8 were originally diagnosed as epidermal cysts with calcification. Only after the series of typical calcified epitheliomas was examined and the epidermal cysts re-studied, was their transitional character appreciated. The significant features were:

(1) a partial layer of squamous epithelium around the periphery, characteristic of epidermal cysts, but showing definite dyskeratosis; (2) cyst contents composed of cornified epithelial cells which presented an unusual degree of preservation of the cytoplasmic outlines; (3) calcification within the cyst contents; (4) rupture of the epithelial lining, with granulation tissue invasion, breaking up the cornified masses into smaller aggregates separated by fibrous tissue.

It is easy to imagine the probable future progression of cases 7 and 8 had there been no surgical intervention. The connective tissue invasion would have progressed to involve the entire contents, breaking it up into irregular smaller masses; the peripheral rim of viable epithelium would disappear; more calcium would be laid down within the keratinized or mummified epithelial masses; the invading connective tissue stroma would become less cellular in time; ossification might or might not develop. If the features already present in cases 7 and 8 progressed in this fashion, there would result in time a picture exactly like that of case 1 or case 5. Yet there can be no doubt that cases 7 and 8 represent epidermal cysts, and can be classified with the calcified epitheliomas only as early and transitional examples.

Case 9. The final example, a mass in the anterior triangle of the neck of 1 year's duration, presented a very different picture from any of the preceding cases. It was classified with the calcified epitheliomas in the literature, but should be definitely separated from the preceding group.

In this tumor there were broad anastomosing ribbons of cells of basal type, forming a convoluted pattern and composed of very densely packed viable nuclei, with numerous mitotic figures. The nuclei were moderately chromatin-rich, with a single nucleolus. No cytoplasmic differentiation was apparent, but the nuclei appeared embedded within a vague symplasmic mass. Figure 3 illustrates this tumor. The significant feature was the presence of squamous cells at some of the margins of these cellular ribbons. The interstices between some of the ribbons were, in turn, filled with masses of mummified squamous epithelium, which appeared to be in relation to the living squamous epithelium. Figure 4 illustrates this feature. In this figure the densely crowded nuclei, clearly of the basal cell type, appear to be those of living cells. Small areas of squamous epithelium are apparent as well as larger masses of mummified cells.

DISCUSSION

It is my contention that the fundamental property of so-called calcified epithelioma is the presence of well preserved masses of squamous-appearing cells, completely non-living, and generally with faint

outlines of nuclear structure. The presence of actual calcification in such masses is not essential, nor is the occurrence of viable epithelium. The question then arises, what is the origin of these mummified masses? The opinion is expressed in the literature that they represent either necrotic or "dyskeratotic" epithelium. It appears from the transitional cases and simple epidermal cysts described herein that, on the contrary, this material is a cornification product, desquamated from a layer of viable squamous epithelium. In the course of the progression, the viable epithelium may disappear to leave only the resistant cornified material. This may or may not become calcified.

In support of this view, the following data may be emphasized. A squamous epithelial layer, especially when lining a cyst but also when covering a free surface, may give rise to several different types of product. A flaky atheromatous material, a definite parakeratosis, a fused and hyaline mass without cell outlines or nuclei, and a cornified tissue preserving the cell and nuclear outlines, all may be seen in different lesions, especially in contents of cysts. Such changes may also be seen in the horny layer of some verrucae vulgares. In these tumors, as in epidermal cysts, several different types of cornified material may be produced consecutively by the same germinative layer. Especially in cyst linings, the still living cells may exhibit degenerative and regressive changes and morphologic alterations which may properly be called dyskeratotic. These changes, apparently an expression of altered growth potencies, may well be precursors of the changes here called mummification.

I have adopted the term "mummified" from the French "momifié," which appears to express most accurately and picturesquely the appearance of the cells. The outlines are preserved. The changes within the cytoplasm are not known, but they are profoundly different from the alterations attending other types of cornification. This is apparent from the different tissue reactions invoked by various types of epidermal cyst contents when connective tissue invasion occurs. Sometimes abundant lipophagic reaction is seen. Lymphocytes, macrophages, polymorphonuclear leukocytes, and foreign body giant cells may be seen in varying proportions. Study of epidermal cysts, as well as of transitional cases 7 and 8, strongly suggests that the various fused and hyaline types of keratinization are quite susceptible to lysis, digestion, and connective tissue invasion. On the other hand, the mummified cell groups seem peculiarly resistant. Although foreign body giant cell reaction is common, no significant lysis is observed. The onset of calcification renders absorption even more difficult, and the resulting lesion may be considered analogous to the persistence of a lithopedion.

In the production of the fully developed quiescent lesion, as in cases 1, 2, 3, 5, and 6, the histogenesis may be as follows. Epidermal cyst contents of varying types, following disappearance of the viable peripheral cell layer, are invaded by organizing connective tissue. The cornified but nonmummified material is absorbed and replaced by connective tissue, but the mummified masses, being resistant to lysis, remain relatively unaltered, and finally calcify. As an alternative, the lining of epidermal cysts may proliferate inward, as seen in Figure 7. Such convoluted epithelial ribbons can become dyskeratotic and give rise to cornified material. If the viable epithelium disappears, as seen so commonly in epidermal cysts, the mummified masses may be very irregularly distributed after connective tissue invasion has replaced the other elements.

Case 9 seems to be a lesion of quite different character. The closely packed viable nuclei of basal cell character strongly suggest a basal type carcinoma. Some portions, however, reveal definite squamous differentiation of almost epidermal character. It seems probable that cells of this latter type give rise to the mummified tissue illustrated in Figure 4. The presence of such material is the sole, but adequate, justification for classifying this case with the others. The lesions described in the literature as recurring are all of this type.

In reference to nomenclature, Côté[3] has pointed out that the term epithelioma, applied to these lesions, is taken from the French, in which language it connotes simply an epithelial growth. The English usage of epithelioma, on the other hand, connotes cancer. That "calcified epithelioma" is a misnomer is generally recognized, but since recurrences and even malignant proliferation have been reported, the term has persisted. In view of the histogenesis expounded in this paper, it is suggested that for the first type of lesion (cases 1 to 8) the term "mummified epidermal cyst" be adopted. Calcification, as well as ossification of the stroma, are accidental features which do not affect the essential nature of the lesion.

Case 9 and the similar cases reported in the literature seem to be of a different category, and may properly be called "basal carcinoma with squamous differentiation and mummification," or, more simply, "basal carcinoma with mummification."

CONCLUSIONS

The term "calcified epithelioma," as used in the literature, has been applied to two clearly separable types of lesions having one feature in common, namely, masses of nonviable squamous-appearing cells of mummified appearance. These mummified masses show clearly de-

fined cell membranes and faint nuclear shadows. There may or may not be deposition of calcium granules, and rarely iron pigment may also be seen. In the first type of "calcified epithelioma," the mummified material is present within a fibrous stroma which may show metaplastic ossification. This type may be traced to simple epidermal cysts through successive steps of dyskeratosis of the lining epithelium, production of cornified material preserving cell outlines and contrasting sharply with the usual contents of epidermal cysts, disruption and disappearance of the epithelial layer and invasion of cyst contents by granulation tissue, resistance of the peculiarly cornified (mummified) material to lysis, and its persistence in the face of vigorous organization elsewhere. Calcification enhances the permanence of the mummified material.

The second type of lesion shows characteristics of basal cell carcinoma, with areas of squamous differentiation and production of mummified keratinized material. Such lesions may recur if operative removal is incomplete.

The term "calcified epithelioma" is a complete misnomer and should be abandoned. For the first type of lesion the name "mummified epidermal cyst" is suggested; for the second, "basal carcinoma with mummification."

REFERENCES

1. Ch'in, K. Y. Calcified epithelioma of the skin. *Am. J. Path.*, 1933, 9, 497–524.
2. Highman, B., and Ogden, G. E. Calcified epithelioma. *Arch. Path.*, 1944, 37, 169–174.
3. Côté, F. H. Benign calcified epithelioma of the skin. *J. Path. & Bact.*, 1936, 43, 575–586.
4. Fevre, M., Huguenin, R., and Paiz, V. Les épithéliomes momifiés ou calcifiés de la peau. *Bull. Assoc. franç. p. l'étude du cancer*, 1938, 27, 355–361.
5. Sutton, R. L., and Sutton, R. L., Jr. Calcifying epithelioma. *Arch. Dermat. & Syph.*, 1935, 31, 48–57.
6. Flarer, F. Cutaneous calcification with special reference to so-called "calcified epithelioma" (nevus ossificans). *Urol. & Cutan. Rev.*, 1931, 35, 284–294.
7. Tanasescu, I., and Balan, N. P. Beitrag zur Lehre von den verkalkten Epitheliomen der Haut. *Ztschr. f. Krebsforsch.*, 1932, 37, 398–410.
8. Huguenin, R., and Perrot, M. Epithéliomes momifiés multiples chez un enfant. *Bull. Assoc. franç. p. l'étude du cancer*, 1939, 28, 627–635.
9. Gromiko, N. Zur Kenntnis der bösartigen Umwandlung des verkalkten Hautepitheliomes. *Virchows Arch. f. path. Anat.*, 1927, 265, 103–116.
10. Warvi, W. N., and Gates, O. Epithelial cysts and cystic tumors of the skin. *Am. J. Path.*, 1943, 19, 765–783.

[*Illustrations follow*]

DESCRIPTION OF PLATES

PLATE 6

FIG. 1. "Calcified epithelioma" (case 1) showing masses of mummified squamous cells with persistence of nuclear shadows, and abundant calcification at the periphery of the masses. The stroma is dense hyaline connective tissue. × 125.

FIG. 2. "Calcified epithelioma" (case 5). The mummified cell masses are invested by a stroma with well formed bone. × 200.

FIG. 3. Basal cell type of "calcified epithelioma" (case 9). The nuclei all stain well with hematoxylin and are entirely viable. × 200.

FIG. 4. Another view of case 9, showing basal type of cell in lower right corner, squamous differentiation in upper right, and mummified squamous material on left. × 500.

Plate 6

King   Mummified Epidermal Cysts

PLATE 7

FIG. 5. Epidermal cyst, with flaky atheromatous type of contents. × 250.

FIG. 6. Epidermal cyst, with papillary type of epithelial lining. The cyst contents exhibit some parakeratotic elements mingled with well preserved cell outlines and inspissated cornified material. The epithelial layer shows dyskeratosis. × 200.

FIG. 7. Epidermal cyst with trabeculated and proliferated epithelial lining. The contents show hyalinized and inspissated material. × 200.

FIG. 8. Case 7. showing cyst contents. Part consists of well cornified cells with preserved outlines and persisting nuclear shadows. forming a mummified tissue. There is a sharp line of demarcation from the granular amorphous contents. presumably representing lysed débris. × 500.

FIG. 9. Case 7. epithelial lining. with dyskeratosis. and mummified desquamation products. × 500.

FIG. 10. Case 4. Mummified material with foreign body reaction. The larger masses are separated by granular débris. (For comparison with Fig. 8.) × 400.

5
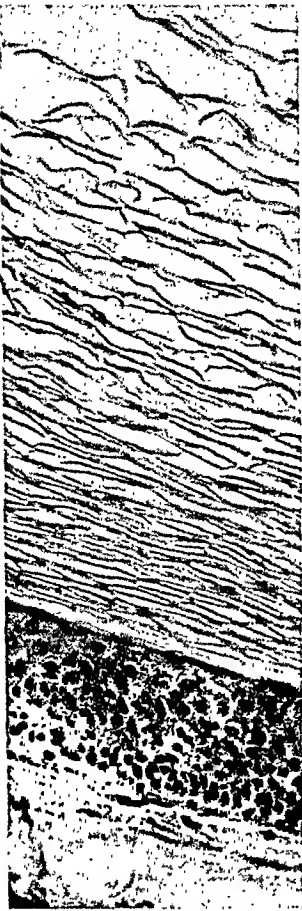
6

7

8
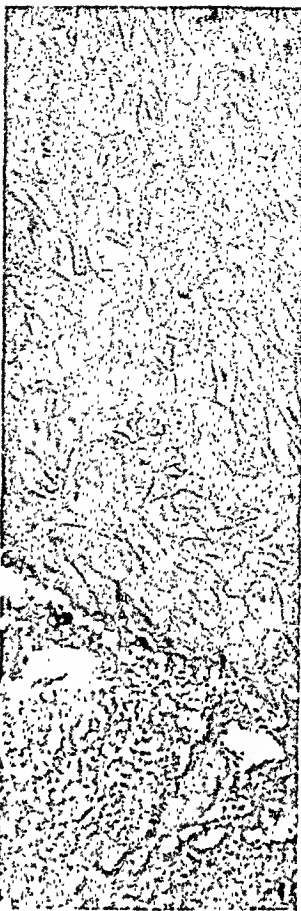
9
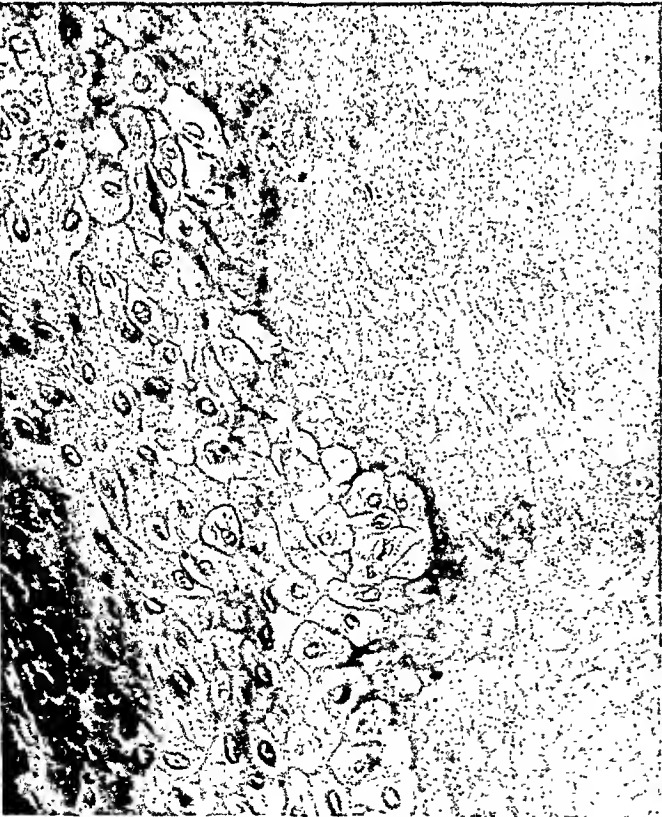
10

EWING'S SARCOMA OF BONE *

Louis Lichtenstein, M.D., and Henry L. Jaffe, M.D.
(*From the Laboratory Division, Hospital for Joint Diseases, New York 35, N.Y.*)

When one studies Ewing's reports of 1921,[1] 1924,[2] and 1928,[3] it is clear that he was attempting to single out among the primary malignant tumors of bone an entity which he first called "diffuse endothelioma" and subsequently "endothelial myeloma" of bone. He thought that the tumor cells were derived from angio-endothelium in the broadest sense. He described the type cell as a small polyhedral cell with pale cytoplasm, small hyperchromatic nucleus, and well defined cell border, and stressed the idea that the tumor cells showed no osteogenic potentialities. He stated that, although tending to be arranged in compact broad sheets, the tumor cells, at least in some places in a given tumor, were often found lying around tiny or larger vascular spaces in "perithelial" arrangement or still circularly, but not around a vessel, in "rosette" formation.

Ewing also consistently stressed certain clinical features as likewise being important in the delimitation of the bone tumor he was discussing. In his opinion, these included: youthfulness of the patients (on the whole); a rather characteristic roentgenographic appearance of the presenting bone lesion; a gratifying initial response of this lesion to radiation therapy; and eventually (in all but a few cases) the appearance of lesions in other bones and especially in the lungs, with fatal result.

However, the concept of Ewing's sarcoma as delineated above has undergone certain criticisms and modifications. Some investigators have even doubted the validity of the basic concept itself, pointing out that it had been founded on, and largely sustained by, clinical and biopsy findings, rather than by study of cases followed through to the end and systematically autopsied. In relation to diagnosis, so much faith has long reposed in the clinical aspects, as sketched above, that, summarized as constituting "Ewing's syndrome," they often took precedence over tissue examination in arriving at a diagnosis—a point of view which has proved itself unjustified. Many, including Neely and Rogers,[4] Swenson,[5] and Barden,[6] have pointed out that evidence is lacking that the roentgenographic picture is sufficiently characteristic for the condition to be of high diagnostic value. Colville and Willis [7] and Willis [8] also emphasized the pitfalls involved in arriving at a diagnosis even through biopsy, and the diagnostic surprise frequently encountered when a case suspected of being Ewing's sarcoma is finally subjected to detailed post-mortem examination.

With due allowance for all these reasons for diagnostic caution, the weight of evidence sustains the existence as an entity of a primary bone sarcoma to which, because of Ewing's pioneer efforts to single it out, the name of Ewing's sarcoma of bone can justifiably be applied. Yet, without prejudice to that central fact, it should be noted that Oberling [9] and Oberling and Raileanu,[10] among others, have dissented from Ewing on the histogenesis of the tumor cells, as well as on the details of the cytology of the tumor. As to histology, Oberling found that the cells of the neoplastic tissue proper tend to appear in a sort of network in which nuclei are prominent, cell cytoplasm is meager, delimiting cell membranes are lacking, and the cells are connected by short or long cytoplasmic processes. He further described the nuclei as uniform in size and generally roundish or ovoid, and as having powdery chromatin and often one or more nucleoli.

On the basis of our own material (17 cases, 4 of which were autopsied) we found that, provided the neoplastic tissue was definitely viable and well fixed and well stained, and irrespective of whether it came from the presenting bone lesion or from a metastatic focus, the appearance of the basic tumor cells corresponded rather to the description given by Oberling than to that given by Ewing. Also, we could not convince ourselves that perivascular orientation of the tumor cells was a characteristic cytologic feature of the tumor in question, although we found that tumor areas which had been heavily invaded by blood vessels, especially in the wake of hemorrhage, often showed tumor cells about capillary spaces or around larger vascular spaces in so-called "perithelial" arrangement. Furthermore, our material failed to provide evidence of the presence of the true rosette (or pseudorosette) formations which have been stressed as part of the cytologic picture of Ewing's sarcoma.

As to histogenesis, Oberling maintained that the tumor develops from the immature reticular cells (the supporting mesenchymal cells) of the bone marrow. For this reason, he conceives and designates Ewing's sarcoma as a reticulosarcoma of bone marrow, and also believes in the kinship (falling short of identity) between reticulosarcoma of bone marrow and the generally recognized reticulosarcoma of lymph nodes. Also, basing his idea on the view that the reticular cells of the bone marrow are totipotent, he maintains that, in some areas of a reticulosarcoma of bone marrow (Ewing's sarcoma), these cells may differentiate in the direction of endothelial cells (cells capable of forming vascular and lymphatic channels) or even in the direction of hemocytoblasts (cells capable of forming myeloid or lymphoid cells). Ewing, taking cognizance of Oberling's views, rejected this conception of the histogenesis of the tumor. Instead, he reiterated, both in 1939 [11] and

in 1940,[12] that the tumor denoted by his name must be conceived as arising from cells which are strictly of angioblastic nature and possess no wider potentialities than the formation of vascular channels.

Like Stout,[13] we saw no evidence in our material that the basic cell of the Ewing sarcoma is totipotent, as Oberling believes, and able to differentiate in the direction of cells capable of forming vascular and lymphatic channels, or even in the direction of cells capable of forming myeloid or lymphoid cells. Like Stout, too, however, we favor Oberling's interpretation of the tumor as a sarcoma of a primitive form of connective tissue and specifically of the mesenchymal supporting framework of the bone marrow. Nevertheless, we feel that designating the lesion as a reticulosarcoma is open to misunderstanding because of the confusingly varied interpretations existing as to the neoplastic potentialities of the mesenchymal reticular framework of the lymphoid and myeloid tissue. Also, it should be pointed out that Parker and Jackson [14] described, under the name of "primary cell sarcoma of bone," a tumor which they hold akin to reticulum cell sarcoma of lymph nodes but distinct from Ewing's sarcoma. For these reasons, and because one might also be thought to believe with Connor [15] that it should be classed as a tumor of the reticulo-endothelial system, we have preferred to use the neutral name of "Ewing's sarcoma" instead of "reticulosarcoma" for the tumor in question.

CLINICAL FEATURES

Age and Sex Incidence and Localization

At the time of admission to the hospital, the 17 patients in our series ranged between 4 and 39 years. However, all but 4 of them were 12 to 19 years of age (these 4 being 4, 8, 24, and 39 years). Correspondingly, the median and average age of the group as a whole were about 16 and 17 years, respectively. It may be that the strikingly narrow age range in our series is favored by the small number of cases, but at any rate our data are in line with the general observation that a very large proportion of the cases in any series (see, for instance, Hamilton [16]) fall within the second decade of life.

Eleven patients (65 per cent) were males, which is in agreement with the experience of most observers that the disorder is slightly more common in males than in females. At any rate, the sex difference in incidence of the disorder is not striking.

Usually, only one skeletal lesion was causing complaint and was demonstrable roentgenographically at the time of the patient's admission to the hospital. In an occasional case, even on admission roentgenographic examination of the rest of the skeleton revealed one or more

additional but clinically silent foci of bone involvement or even pulmonary metastases. However, in connection with localization, we shall refer only to the presenting lesion—the one giving rise to the complaints which brought the patient to the hospital.

In 12 of our 17 cases, the presenting lesion was in one of the bones of the trunk. Specifically, this bone was an ilium in 4 cases, an ischium in 2, a pubis in 1, a rib in 2, a scapula in 1, a clavicle in 1, and a vertebra in 1. In the remaining 5 cases, the presenting lesion was in a long bone, and, specifically, it was in a humerus in 2 cases, a femur in 2, and a fibula in 1. Why, in our cases, the presenting lesions were preponderantly in bones of the trunk we do not know, but this has been true, also, in the experience of others.

History of Trauma

In only 5 of our cases was there a history of definite, fairly recent trauma to the site in which the presenting tumor developed, the trauma having antedated the discovery of the tumor by 1 to 6 months. As to the other cases, 1 patient stated that he had twisted his ankle 4 years before admission for a tumor in the fibula, but the actual complaints which had brought him to the hospital were of only 1 year's standing. Two patients implicated functional trauma of very recent occurrence: handball playing by one and broad-jumping by the other. In 2 additional cases, the site of the trauma mentioned by the patient was near, but not the same as, the site of the presenting tumor. In the remaining 7 cases, the patients gave no history of antecedent trauma to the region of the presenting lesion.

From these data, it is difficult to draw any conclusion as to a possible causal relation of trauma to Ewing's sarcoma. The 5 cases in which a definite history of possibly relevant antecedent trauma was recorded are counterbalanced by the 7 in which there was no history of such trauma. In the remaining 5 cases, the weight of the evidence is against the significance of the trauma, since the trauma was merely a mild functional one, or was not at the site of the subsequent tumor, or preceded the appearance of the tumor by an implausibly short period. Certainly, the data constitute no overwhelming evidence in favor of a causal relation between trauma and Ewing's sarcoma.

Clinical Complaints and Findings

Survey of the clinical histories of the patients in our series shows that local (and/or referred) pain was the one consistent complaint. With few exceptions, the pain was of at least some months' standing, and in several cases it had been present for at least 1 year before admission. Usually, also, it had become increasingly severe and persistent

during some weeks or months immediately before admission. With the local pain, there were often complaints related to spread of the tumor beyond the limits of the bone and varying with the location of the presenting lesion. Thus, for instance, from patients in whom some part of an innominate bone was involved, there were usually complaints of disability relating to the hip joint and sometimes also of radiating pain down the lower limb. In connection with presenting lesions near the end of a long bone, there were sometimes complaints of lameness or stiffness of the corresponding joint, and, in one case in which the lesion was near the lower end of the femur, there were repeated serous effusions into the knee joint. In the cases in which the presenting lesion was in a lumbar vertebra, there were, in addition to the local pain, complaints ascribable to implication of nerve trunks in the area, such as pain radiating down the limbs, and tingling sensations and weakness in the latter. Location of the presenting lesion in a rib was found associated with pleural effusion in one case. Other locations of the presenting lesion (for instance, in the skull) are associated with their own special clinical disabilities.

Just as local pain was the dominant clinical complaint, so the presence of a local tumor mass was the dominant clinical finding at the time of admission. On admission, a more or less prominent tumor mass was palpable at the site of the presenting bone lesion in all but 3 of our 17 cases. This fact indicates the strong tendency of Ewing's sarcoma to break out through the cortex of the bone and spread in the surrounding tissues. Notably large tumor masses were palpable in some cases in which the tumor appeared in an innominate bone. Spreading internally toward the pelvic cavity, the tumor beyond the limits of the bone could then sometimes be palpated as an elastic, irregular, globular mass, through the rectum if the tumor was low down, or in the lower quadrant of the abdomen if it was higher up. Spreading externally, a tumor springing from an innominate bone sometimes produced a large tumor mass palpable in the groin or in the gluteal region. In one of our cases in which the presenting lesion was in the shaft of a humerus there was likewise a very large extra-osseous tumor mass connected with the bone. When the presenting tumor was in a superficially located bone such as a clavicle or a rib, the mass produced by extra-osseous spread could be seen as well as palpated.

Tenderness to pressure at the site of the lesion was recorded in practically all cases. Frequently, the subcutaneous veins overlying the presenting lesion were found to be prominent. However, it was only exceptionally that increased local heat was mentioned in connection with the physical examination.

A survey of the temperature charts and the laboratory findings in

our cases revealed what appeared to be significant information of clinical value. Many of the patients were in the hospital for almost a week before specimens were secured for biopsy. During this time they had a slight fever, with daily rises in temperature to about 101° F.* These patients generally presented a secondary anemia (with a red blood cell count of about 3,500,000), and sometimes also a leukocytosis. In addition, they usually showed a high sedimentation rate of the blood. Taken together, these findings proved to be more significant in respect to the immediate prognosis than the size of the presenting lesion. Specifically, the cases in which some fever and secondary anemia, together or even alone, were noted ran a fulminating course, ending in death within a few months after admission to the hospital. On the other hand, those patients who had no fever on admission, and no anemia or increased sedimentation rate, tended to survive for a year or more after admission.

ROENTGENOGRAPHIC FEATURES

Roentgenographic Appearance of the Presenting Bone Lesion

By the presenting bone lesion we mean, as already indicated, the one causing the complaints which led the patient to enter our hospital. This was often the only lesion discernible even when the entire skeleton was roentgenographed on admission, and in any event it was the one which guided roentgenographic diagnosis. One need only review the presenting lesion in a series of cases to appreciate the difficulty of making a diagnosis of Ewing's sarcoma by x-ray examination alone. If the amount of bone involvement in the presenting lesion roentgenographically is still small and no lesions are found elsewhere, the picture may be misconstrued as an inflammatory lesion. However, in most cases the picture of the presenting lesion suggests a malignant tumor, although often misinterpreted as some malignant tumor other than Ewing's sarcoma.

---

* One case presented a diagnostic problem not only because of a definitely febrile course but because of atypicalness of the local clinical complaints and findings. The patient was admitted to the hospital complaining of difficulty with the right knee joint, dating back 8 months. The knee was painful, warm, tender, and swollen from the presence of fluid in it. Some rarefaction of the cortex and spongiosa of the medial condyle of the femur was noted roentgenographically, but there was no clear-cut indication of the presence of a tumor in the bone or overlying soft parts. The clinical impression was that the condition of the knee joint proper and of the condyle had its basis in some infection, as indicated by the fact that the patient had repeated bouts of fever, sometimes reaching 103° F., and that serous fluid could be aspirated again and again from the joint. Repeated bacteriologic studies of the joint fluid, and various agglutination tests on the blood failed to give evidence of infection. However, in the ensuing 5 weeks there was progressive destruction of the medial condyle, and the assumption of an infectious lesion became less tenable. Biopsy revealed Ewing's sarcoma of the lower end of the femur, and the limb was disarticulated. The patient died 5 months after admission, and at autopsy widespread metastases were found.

The only fairly consistent roentgenographic finding is evidence of lysis of bone, by itself a rather nondescript feature. Thus in some cases the presenting lesion may appear merely as a small zone of mottled rarefaction reflecting destruction of the spongiosa and, to a lesser degree, of the overlying cortex, associated with what is as yet only a trace of periosteal new bone apposition in reaction to the neoplastic tissue which has penetrated beyond the cortex. This picture (which may also include some areas of condensation) is very likely to suggest an inflammatory lesion (pyogenic or tuberculous osteomyelitis) rather than a tumor, but within a month or so the roentgenogram presents evidence of rapid extension of the pathologic area within and beyond the bone, now strongly supporting a diagnosis of malignant neoplasm. Although the roentgenogram still shows only a relatively small area of bone destruction, this cannot be taken as indicating the actual extent of involvement of the bone, the marrow spaces of which may already be riddled by neoplastic tissue (Figs. 1, 2, and 9).

When the initial roentgenograph of the presenting lesion shows rather clearly that one is dealing with a malignant tumor, one usually notes a large area of bone destruction, often with a large overlying soft-tissue mass. The affected area in the bone may show distention of its outline, but, if present, this is not pronounced. However, the affected area appears irregularly rarefied and mottled from the presence of smaller or larger foci of relative radiolucency and shows disruption of the cortical outline over a variable region. From a series of cases it can be stated that reactive deposition of new bone by the periosteum, where the neoplastic tissue is penetrating the cortex, is certainly not conspicuous. When, as is commonly the case, Ewing's sarcoma involves bones other than long bones, evidence of periosteal new bone apposition, although not uncommon, is not a striking finding (Figs. 3 and 4). It may not even be such in connection with involvement of long bones. When the shaft of a long bone is the site of Ewing's sarcoma, one does not commonly observe the concentric onion-skin-like layers of periosteal new bone of a laminated pattern held to be so characteristic of the roentgenologic appearance of this tumor. Rather, a substantial portion of the shaft may show irregular mottled rarefaction, perhaps with complete absence of significant periosteal bone apposition (Fig. 5). In one of our cases of Ewing's sarcoma of the shaft of a humerus, there was considerable onion-skin-like periosteal new bone apposition, but this was not circumferential, being limited to the lateral surface, while on the medial side the bone was overlaid by a thick mass of neoplastic tissue which had broken out from the interior of the bone. In this case (Fig. 6), on the basis of the roentgenologic appearance, the

lesion was regarded clinically as an osteogenic sarcoma without evidence of exuberant new bone formation (that is, as a so-called "osteolytic" osteogenic sarcoma). In another case of Ewing's sarcoma of a humerus, the roentgenogram of the affected portion of the shaft showed an irregular moth-eaten appearance of the cortex without thickening, associated with more or less transverse streaks of radiopacity in the soft portion of the tumor mass overlying the outer surface of the bone. Again the lesion was interpreted clinically as an osteogenic sarcoma (Fig. 7).

Altogether, the only conclusions that can be drawn in regard to the roentgenographic appearance of the presenting lesion are that bone destruction (osteolysis) is the dominant feature of Ewing's sarcoma and that there is no typical appearance for this lesion. In general, Ewing's sarcoma is a tumor difficult to diagnose on a roentgenographic basis, often being mistaken in its early stage for an inflammatory lesion and in later stages for malignant tumors of other nature, including metastatic neoplasms. In many cases it may be quite difficult to make a differential diagnosis, on a roentgenographic basis, between Ewing's tumor and chondrosarcoma, "osteolytic" osteogenic sarcoma, malignant lymphoma, or metastatic neoplasms (including metastatic neuroblastoma). Sometimes, too, a solitary lesion of eosinophilic granuloma may be mistaken for Ewing's sarcoma. To make certain that a suspected tumor is Ewing's sarcoma, tissue examination is essential, but it cannot be emphasized too strongly that a pathologist confronted by a specimen taken from a suspected case for biopsy may easily be mistaken in his opinion on this basis also, especially if the tissue available is meager. However, in this connection, the error is more likely to be that of misidentifying other lesions (anaplastic carcinoma, metastatic neuroblastoma, malignant lymphoma) as Ewing's sarcoma than the reverse.

Roentgenographic Appearance of the "Metastatic" Bone Lesions

Whether the additional lesions found roentgenographically on admission or subsequently, represent metastases from the presenting lesion or are independent primary growths does not concern us precisely here. Roentgenographically, these additional lesions, like the presenting lesion, show evidence of lysis of bone. They appear first as rather faint, slightly mottled areas of rarefaction. As the resorption of the bone increases, the small, multiple, roundish foci of rarefaction become more distinct and may merge into larger, more clear-cut areas of radiolucency. In flat bones, such as those of the skull or the ilium, multiple, clear-cut, punched-out areas of rarefaction may appear in

consequence of lytic destruction of the spongiosa and overlying cortex (Fig. 8). Even a neoplastic fracture of a long bone from destructive resorption may become manifest. It is important to bear in mind, however, that the actual extent of involvement of the skeleton at any one time is never adequately reflected roentgenographically. This is true even in fatal cases in which a number of destructive lesions have been demonstrated roentgenographically in bones other than the one containing the presenting lesion. At autopsy, if many additional bones are opened, they, too, will be found to have been far more extensively invaded than was suspected from roentgenographic study of the skeleton shortly before death.

Morphologic Features

Gross Description

Our experience supports the idea that Ewing's sarcoma arises in the marrow spaces of the interior of the affected bone, rather than in the haversian spaces of the cortex or beneath the periosteum. Also, as has been indicated, anatomic examination of an affected bone will reveal much more extensive involvement than the roentgenographic or clinical findings would suggest. This can be effectively demonstrated in cases in which the presenting lesion is in a long bone, which is made available by amputation. Roentgenographically, in a femur from such a case, the disease seemed to affect only the medial condyle and the adjacent part of the shaft. The cortex in this region was fuzzy and had a superposed soft-tissue swelling, about 2 cm. in thickness. When this femur was stripped of its surrounding muscles and cut in the frontal plane, it showed neoplastic tissue not only in the medial condyle but also in the lateral condyle and contiguous portions of the shaft, in the major marrow cavity, and even in the marrow spaces of the spongiosa of the upper end. The neoplastic tissue in the region of the medial condyle and that which had penetrated beyond the cortex in this region was, for the most part, discolored by hemorrhage and interspersed with yellowish areas due to necrosis of both neoplasm and spongiosa. Elsewhere, for the most part, the neoplastic tissue was not modified by hemorrhage or necrosis, was whitish, and, notably in the major marrow cavity, took the form of massed, soft, glistening tumor nodules. Thus the diseased area clearly visible in the pre-amputation roentgenogram of this femur was merely the area in which the changes were most advanced and destructive (Figs. 1, 9, and 10). Neither the inguinal nor the popliteal lymph nodes were enlarged or involved by tumor. Furthermore, in spite of the extensive implication of the femur,

no tumorous involvement was discernible in the tibia, fibula,. or bones of the foot, all of which were opened and examined. These negative findings are of interest because at autopsy, $3\frac{1}{2}$ months after the disarticulation of the right lower limb, widespread involvement of the skeleton and visceral metastases were found.

In contrast to a small tumor mass beyond the limits of the bone proper, as demonstrated in the femoral lesion just described, one finds, relatively often, a very large tumor mass beyond the limits of the bone, as part of the presenting lesion. This was well demonstrated in the case of a young girl whose presenting lesion was in the left ilium and whose complaints were of only a few days' standing at the time of admission to the hospital, but who then showed a firm tumor mass of the size of a neonate head, fixed to the ilium and palpable in the lower quadrant and groin. Although this patient appeared to be in good general health on admission, roentgenograms showed pulmonary metastases, and she died 4 months later. So far as the presenting lesion was concerned, autopsy revealed a huge mass overlying the inner surface of the left innominate bone, which had pushed the urinary bladder and the genital organs anteriorly and the sigmoid and rectum medially. On removing the left innominate bone, it was found that the tumor had extended posteriorly through the ilium and was bulging into the gluteal muscles and penetrating the capsule of the hip joint. The extra-osseous neoplastic tissue was soft, friable, extensively hemorrhagic, spongy and cystic on the whole, and in many places almost diffluent. The iliac bone was riddled by neoplastic tissue which was cystic in many places and there were many defects in its cortex, both on the inner and outer aspects, from which, as noted, the tumor had spread beyond the limits of the ilium proper into the surrounding soft tissues (Figs. 3 and 11).

As previously mentioned, 4 of the 17 cases upon which this report is based were autopsied. Two of the autopsies were performed by us. The other two were performed at Montefiore Hospital, New York City, and we are indebted to Dr. Samuel H. Rosen of the Laboratory of Pathology of that hospital for the opportunity of studying the protocols and slides. These autopsies were carried out with full awareness of the general lack of thorough autopsy studies in cases of Ewing's sarcoma.

At the time of death, 2 of these patients were 16 years of age; the other 2 patients were 18 and 19 years old, respectively. Three were females. The youthfulness of these patients makes it improbable that the skeletal lesions represented metastases from a carcinoma, although the possibility cannot be excluded. If we were dealing in these cases with metastases of carcinoma from an unrecognized primary growth,

whatever its site and however anaplastic the metastases might be, it is highly improbable that these lesions would consistently present the cytologic pattern peculiar to well preserved Ewing's sarcoma, to wit: cells of rather uniform size, with ill defined borders, little cytoplasm, and fairly large and rather uniform roundish or oval nuclei showing scattered chromatin.

Also, the findings, at least in the 2 cases (one male and one female) which we personally autopsied, clearly ruled out the presence of a hidden carcinoma. The breasts and testes were carefully searched for neoplastic tissue and none was found. In both cases, the lungs were given particular attention. In one no grossly visible tumor nodules were found anywhere in the pulmonary parenchyma. The bronchi, which were opened to the very small branches, as well as the hilar lymph nodes showed no gross evidence of involvement, reducing the likelihood that we had overlooked a primary bronchial carcinoma. In the other case, although all lobes of the lungs were riddled with hundreds of soft, richly cellular, largely hemorrhagic and liquefied tumor nodules of various sizes, they showed no single massive tumor, and it was plain from the gross appearances that the lesions were metastatic. In the course of the autopsies, full consideration was given to the fact that the gastrointestinal tract, and especially the stomach, can be the site of unsuspected carcinoma, but neither the gastrointestinal tract nor the lymphoid tissue regional to it showed tumorous involvement. Every precaution was taken to exclude the possibility that one was dealing not with a tumor primary in the skeleton but merely with metastases to the skeleton from a carcinoma which, in its primary site, was overlooked because it was inconspicuous or, although observed, was misinterpreted as a metastasis.

In a case thought to represent Ewing's sarcoma, the problem posed by neuroblastoma (primary in the adrenal medulla or in sympathetic nervous tissue elsewhere) is an even greater challenge than that raised by carcinoma. Sympathicoblastoma must always be ruled out in such a case, since this tumor not only has a strong tendency to metastasize widely to the skeleton but may bear a confusing cytologic resemblance to Ewing's sarcoma. This point was rightly stressed by Willis,[8] although he has often been misinterpreted as rejecting entirely the entity of Ewing's sarcoma and holding that all such cases represent merely metastases, particularly from neuroblastoma. Be that as it may, the adrenals in all 4 of our autopsied cases failed to show, on detailed gross examination, evidence of tumorous involvement or of any other abnormality. In the 2 cases which we autopsied personally, an extended search of the areas around the adrenals and of the sym-

pathetic chains along the vertebral column failed to show evidence of an extra-adrenal sympathicoblastoma.

As to the viscera, we have already pointed out that the lungs grossly may be found free of neoplastic tissue or, on the contrary, riddled with metastatic nodules. Under the latter conditions, we also have found the parietal pleura studded with tumor masses, some of which were large and fungating. In 2 cases, the liver presented numerous metastases, mainly in the form of nodules a few millimeters to somewhat more than a centimeter in diameter. In one case or another, metastases were noted in one or more of the following organs: heart, spleen, kidneys, pancreas, and thyroid. Finally, it should be noted that the lymph nodes, by and large, tended to be free of neoplastic tissue, although in one case some of the paravertebral and pelvic lymph nodes showed, microscopically, some nests of tumor cells in the peripheral sinuses as extensions of the neoplasm from the underlying vertebrae. The striking lack of involvement of the lymph nodes is additional evidence against the possibility that an occult primary carcinoma or neuroblastoma was present.

As already indicated, one can expect to find at autopsy that much of the skeleton, in addition to the bone with the presenting lesion, is affected, and much more extensively than one would have suspected from the ante-mortem roentgenographs. The question which cannot be answered definitely is whether the wide dissemination through the bones represents metastatic spread of the neoplasm or its autochthonous appearance in multiple sites.

At any rate, the calvarium is likely to show the neoplasm permeating the diploic spaces, and, in addition, areas in which neoplastic tissue has eroded or completely destroyed the tables. In the latter case, the calvarium will show actual defects, frequently several centimeters in diameter, filled with cellular, gray white or even greenish yellow neoplastic tissue which may elevate or even penetrate the regional calvarial coverings. The marrow spaces of the ribs and sternum, too, are likely to be filled with neoplasm, and thinning and erosion of the cortex may be associated with focal masses of neoplastic tissue beneath the periosteum. In both cases which we autopsied, large sections of the vertebral column were removed, and here too we found the marrow spaces of the bodies, arches, and spinous processes extensively infiltrated. In one case in particular, practically every dorsal and lumbar vertebral body showed areas in which the neoplasm in the marrow spaces and the supporting spongy bone appeared yellowish in consequence of necrosis (Fig. 12). Where there was no necrosis, the neoplastic tissue was grayish, soft, and obviously cellular. In many places the neoplastic

tissue was extending through the bodies and beneath the anterior vertebral ligament. From the third dorsal segment to the first lumbar segment, the new growth had also extended beneath the dura, narrowed the spinal space, and cuffed and compressed a large section of the spinal cord, with resultant degeneration of the latter.

Microscopic Description

Although Ewing's sarcoma does have a characteristic cytologic pattern (as Melnick [17] also maintained), secondary changes may obscure it or make it difficult to demonstrate in an individual specimen taken for biopsy, even if it has been obtained by surgical incision. Thus, a specimen may show large fields in which the appearance of the individual tumor cells has been altered by degeneration and necrosis, areas in which the neoplastic tissue as a whole has been modified by hemorrhage and reparative reaction to it, and even areas in which reactive inflammatory changes dominate the picture. It is because such secondary changes are not relegated to the background that the reputation of Ewing's sarcoma for variability and inconstancy of its cytologic pattern in biopsy specimens from case to case has developed and persists.

However, secondary changes in the neoplastic tissue do not present the only difficulty with which one is confronted in attempting to make a diagnosis of Ewing's tumor on the basis of a biopsy specimen. The diagnosis "Ewing's sarcoma" often has become a mere refuge when one is confronted by a puzzling malignant tumor in a bone, and is likely to be applied rather loosely and by default of a better opinion unless one's anatomic conception of Ewing's sarcoma is definite. This has been discovered by others, too, when they have re-evaluated their cases. It was brought home to us by restudy of all the material (27 cases) listed in our files under the heading of Ewing's sarcoma during the past 20 years. Among the cases so listed, there were some in which the material was so poor in well preserved tumor cells that we would now hesitate to make the diagnosis of Ewing's sarcoma on that basis alone. Although some of the lesions may have been Ewing's sarcomas, we must have been largely guided by the clinical, and especially the roentgenographic, findings in arriving at that diagnosis. There were also a number of cases in which restudy showed that our original diagnosis of Ewing's sarcoma had been incorrect, and, specifically, that we had erroneously included under this diagnosis some cases in which the bone lesion under consideration was actually a myeloma, a lymphocytic lymphoma, an anaplastic metastatic carcinoma, or a metastatic neuroblastoma. After excluding all cases in which the available tissue was defective in quality or inadequate in amount and those in which the

original diagnosis now seems clearly to have been erroneous, there remained the 17 cases upon which the present report is based.

The characteristic cytology of Ewing's sarcoma, irrespective of the source of the neoplastic tissue, is manifested through the presence of smaller or larger fields of tumor cells which lack clearly delimited cell boundaries, the nuclei being crowded together and of fairly uniform appearance. These nuclei are round or ovoid, are about twice as great in diameter (or, in the case of the ovoid ones, perhaps three times as great in the longer axis) as the nucleus of a lymphocyte, and have finely divided or powdery chromatin and often one or more nucleoli. As a rule, the individual nuclei appear enmeshed in, and slightly separated by, a loose, more or less vacuolated cytoplasmic fabric. In some fields, however, they may be found crowded together (perhaps to such an extent that many of them are even pressed into an oval shape), and in such fields there is but little cytoplasm between them. It should also be noted that in the fields presenting the general cytologic picture just described, vascularity is usually not a prominent feature (Figs. 13 and 14).

Cellular areas showing the characteristic cytology described above have to be searched for in the specimen taken for biopsy from the presenting bone lesion in an individual case, since the neoplastic tissue may have undergone abundant secondary changes, such as degeneration and necrosis. Degeneration is indicated in the nuclei by pyknosis and reduced size, and such nuclei are likely to be surrounded by a narrow zone of cytoplasm with a delimiting cell border. It is such cells that approximate most closely the picture given by Ewing for the type cell, to wit: a small polyhedral cell with pale cytoplasm, a small hyperchromatic nucleus, and a well defined cell border. Intermingled with the fields in which the tumor cells are undergoing degeneration there are usually areas in which the cells have undergone necrosis. Degenerating, but particularly necrotic, neoplastic tissue may, in some places, be heavily infiltrated by polymorphonuclear leukocytes (Fig. 15). Hence, if only a limited fragment of tissue is examined and sufficient care is not taken, this picture may be misinterpreted as representing an infectious process rather than a tumor. In connection with the presence of polymorphonuclear leukocytes in degenerating and necrotizing neoplastic tissue, we have found no evidence in favor of the concept once suggested that Ewing's sarcoma usually arises in marrow previously altered by fibrosing or sclerosing osteomyelitis.

Free hemorrhage into fields of neoplastic tissue, especially if it is extensive, comes to be associated with the ingrowth of many blood

vessels into those fields. If the neoplastic tissue in these areas is not necrotic, one will note that many of these vessels are collared by tumor cells. However, the vessel spaces are not lined by tumor cells, and between the latter and the lining cells there is tissue representing the wall of the vascular space. Thus, while it is true that in such fields one does see tumor cells about capillary spaces or around larger vascular spaces in a so-called "perithelial" arrangement, one does not see this orientation of tumor cells to any pronounced extent except in connection with hemorrhage. It is on this account that no distinctive cytologic significance attaches to such findings. Similar perivascular orientation of tumor cells is observed in connection with sarcomas of other kinds in which focal areas have undergone extensive hemorrhage (Fig. 16).

We turn now to the question of the presence of rosette (or pseudorosette) formations. In connection with an occasional Ewing's sarcoma, authors have reported and illustrated formations in which cells are arranged circularly (although not around a vessel) in so-called rosette formation. However, in such illustrations it can be seen that the centers of these formations represent degenerated cells with shadows which are still perceptible, rather than fibrillar or granular cores as in neuroblastoma. We think that the "pseudorosettes" illustrated by Gharpure [18] in his case of Ewing's sarcoma clearly show that the core about which the viable tumor cells are circularly disposed represents a mass of necrotic tumor cells, the outlines of which are still plainly visible. Also, the "rosettes" illustrated in the case presented by Foote and Anderson [19] seem likewise to appear in tissue fields where cells are undergoing necrosis. In our own material, we also have occasionally encountered a tumor field in which viable tumor cells were disposed about cores of degenerating cells. In one case this was a rather prominent feature, but even then the formations were not clearly suggestive of the rosettes of neuroblastoma (Figs. 17 and 18).

Finally, we come to the question of reticulum fibrils in Ewing's sarcoma. It appears that these are not a consistent nor a prominent feature of the histologic picture. Indeed, there is considerable variability in regard to these fibrils, from lesion to lesion and even from part to part of the same tumor section. Some of the lesions, in part or throughout, have at most only a few stray argyrophil fibrils in an entire low-power field. Other lesions show more numerous fibrils, but even in them the fibrils are irregularly distributed and are seen only between smaller and larger groups of tumor cells. In no tumor did we regularly see large fields of tissue showing a lattice or meshwork of reticulum fibrils outlining not merely cell groups, but the individual

tumor cells. In view of this variability, it is clear that there is no characteristic histologic pattern for Ewing's tumor in so far as these fibrils are concerned.

THE PROBLEM OF PRIMARY RETICULUM CELL SARCOMA OF BONE IN RELATION TO EWING'S SARCOMA

In 1939, in an article entitled "Primary Reticulum Cell Sarcoma of Bone," Parker and Jackson [14] called attention, on the basis of 17 cases, to a malignant bone tumor which they held to be distinctive and, in particular, to be different from Ewing's sarcoma, with which it is most often confused. They believed this tumor to be derived from the reticulum cells of the marrow of the affected bone, and indicated that the cell type of this tumor was identical with that of reticulum cell sarcoma of lymph nodes and other tissues, and that diagnosis of the condition must rest upon tissue examination. As to the histology of the tumor, they stated that the cell nucleus, which is from one and one-half to two times larger than in a lymphocyte, varies in shape from round to oval; frequently, it may be indented or lobulated. The chromatin may be finely divided and scattered, or, on the other hand, it may be coarser and nucleoli may be present. There may be considerable cytoplasm about the nucleus. Evidence of ameboid activity, as indicated by the oval or elongated shape of the cell and its nucleus, is frequently present and is a characteristic feature. Binucleate forms occur, but true tumor giant cells do not. Mitotic figures are often present in large number. When the neoplastic tissue is stained in order to bring out the reticulum, the latter is found to run in delicate threads and strands around groups of tumor cells and also between individual cells.

Thus, on cytologic grounds, there seem to be some tangible, although not striking, differences between the tumor described by Parker and Jackson [14] and Ewing's sarcoma. Stout,[13] however, found himself unable to distinguish between the two even on a cytologic basis, holding that they are simply variants of the same tumor. On the other hand, it is evident from the revised (1939) classification by the Registry of Bone Sarcoma of the American College of Surgeons that Ewing [11] accepted the concept of primary localized reticulum cell sarcoma of bone as a tumor entity, and equally evident that he held it to be distinct from the Ewing tumor. The article by Edwards [20] supplied further support for the concept of primary localized reticulum cell sarcoma of bone and gave details of a case which was followed through to autopsy. The article by Gall and Mallory,[21] although devoted to the problem of malignant lymphoma in general, likewise sustained this

thesis and pointed out that sometimes a malignant lymphoma arises as a solitary lesion in the marrow of a single area of bone, and tends to remain localized there for a long time before spreading even to the adjacent lymph nodes. In particular, Gall and Mallory indicated that among the relatively infrequent instances in which a malignant lymphoma takes this clinical form, cases of clasmatocytic lymphoma (one of their two varieties of so-called reticulum cell sarcoma) are prominent. In their Table IV (page 404), they list 6 cases of clasmatocytic lymphoma which appeared to be initially localized to a bone.

Clinically, a primary reticulum cell sarcoma of bone presents itself as a painful, destructive lesion, often extensive, but localized to the area of bone in question, while the general health of the patient is good. When it is a long bone that is affected (as is most often the case), the end and much of the shaft are usually involved, and a neoplastic fracture may have resulted. The roentgenographic picture is not distinctive, showing only that one is dealing essentially with an osteolytic lesion, which may have broken through the outer bounds of the cortex and extended into the surrounding soft parts. In this respect, the roentgenographic picture is interchangeable with that of Ewing's sarcoma. As to treatment, Parker and Jackson [14] stressed the practical value of early diagnosis (by biopsy) followed by immediate amputation or ablation if the lesion is in a site permitting this, and otherwise by wide local excision if possible. They further advocated that this should be followed by local radiation therapy, but pointed out that such therapy by itself is inadequate. Of the 17 patients whose cases are discussed by them, 7 who received appropriate treatment have been apparently free from tumor for 10 years or more. In several of the other cases death supervened, but in none of these was an autopsy done. However, there were indications that late in the course of the disease the tumor may extend to the regional lymph nodes and even spread distantly by way of the blood stream.

It is a knotty problem to decide whether, and if so to what degree, one should differentiate between primary reticulum cell sarcoma of bone and Ewing's sarcoma. Certainly, in view of its reliable sponsorship, the concept of reticulum cell sarcoma of bone should not be lightly pushed aside. If further clinical experience supports the current observation that primary reticulum cell sarcoma of bone has a definitely more favorable prognosis (if correctly and promptly treated) than Ewing's sarcoma, there will be a good practical reason, also, for preserving the distinction. If, in a given case, the cellular structure favors a diagnosis of reticulum cell sarcoma of bone and there is as yet no clinical evidence of distant spread of the tumor, one would be all

the more justified (when the lesion is in a suitable site) in urging prompt amputation or ablation of the affected part.

THE PROBLEM OF NEUROBLASTOMA WITH SKELETAL METASTASES IN RELATION TO EWING'S SARCOMA

The fact that a sympathetic neuroblastoma (sympathicoblastoma) commonly metastasizes to bones has been known for a long time. Hutchison,[22] and Tileston and Wolbach [23] have pointed out that one can anticipate finding, at least at autopsy, a malignant adrenal tumor as the primary lesion in infants and children clinically presenting tumorous involvement of cranial bones, associated with proptosis from tumorous involvement of the orbital region and tumorous enlargement of the preauricular and other regional lymph nodes. From the cases reported by these authors, and from those which they collected from the literature (cases now assignable to adrenal neuroblastoma), it was evident that metastases to bones other than those of the skull are also often found, and that metastases to the liver, kidneys, and lymph nodes in general were among the other common findings.

Further progress in the understanding of sympathetic neuroblastoma has revealed that, although the adrenal medulla is the most common site of origin for these tumors, it is by no means the only one. Cases have been reported in which they arose from some part of the sympathetic nervous tissue elsewhere in the body, notably from the sympathetic chains, but sometimes even from the sympathetic tissue of organs. While infants and young children are the most common victims, occasional instances have been reported in which sympathetic neuroblastomas developed in adults. Also, it has become clear that in so far as the skeleton is concerned, the clinically presenting, destructive bone lesion (if there is one) may be in a long bone or some bone other than the skull.

In respect to cytology, Tileston and Wolbach [23] stressed the diagnostic significance, for the condition in question, of the finding (in various numbers) of tumor cells arranged in rosettes. It was Wright [24] who pointed out that these tumors take their origin from the pluripotential cells of the sympathetic nervous system, and that the rosettes are ball-like aggregations of tumor cells enclosing a small central meshwork of filamentous neurofibrils, some of which can be seen to constitute processes of the cells making up the periphery of the rosette. In addition, he pointed out that, aside from rosettes, one may be able to find, as also peculiar to neuroblastoma, masses of tumor cells interspersed with and penetrated by fibrils running parallel in bundles. But the demonstration of neurofibrils, either in parallel bundles or as a meshwork in the center of the rosettes, may be difficult. Specifically,

in a given case, few fibrils may have been laid down, or, by degeneration or post-mortem change, such fibrils as were laid down may have become transformed into hyaline or granular material and be difficult to demonstrate on this account. This is especially true of the fibrils of the rosettes. Under such circumstances, whatever rosettes are present appear as formations in which several rows of cells surround a finely granular, eosin-staining mass without a central lumen.

In a particular case, rosettes may be fairly numerous in both the primary growth and the metastases, conspicuous in the primary growth and sparse in the metastases, or difficult to find in either. As to the type cell of the tumor, there are differences from lesion to lesion, depending on the predominating level of maturation. In the most primitive type, the dominating cell maintains the lymphocytoid character of the parent stem cell. This cell is thus a small round cell (strongly resembling a small lymphocyte) with a dense hyperchromatic nucleus practically filling the entire cell so that there is little cytoplasm. Some of the cells, although maintaining this general character, may be oval, while others, especially at the periphery of the rosettes, may be piriform. In more differentiated sympathetic neuroblastomas, the cells, although mainly round, are distinctly larger than those just described and may have vesicular nuclei and a clear ring of cytoplasm about the nucleus, and even some cytoplasmic processes. In still further matured neuroblastomas, some tumor fields may even show sympathetic ganglion cells.

Against this background, we are in a position to understand Willis' point of view on neuroblastoma in relation to Ewing's sarcoma. Prior to the publication of the first relevant article by Colville and Willis [7] in 1933, it seems not to have been adequately stressed that care must be taken to exclude the possibility that one may be dealing with a sympathetic neuroblastoma metastatic to the skeleton in cases supposedly representing Ewing's sarcoma of bone. In that article a case is detailed (as is another one in 1940 [8]) in which there was a presenting tumor in a femur which had the usually accepted clinical and roentgenographic characteristics of Ewing's sarcoma. In these cases the clinical course, and in particular the susceptibility of the tumor to radiation therapy, seemed to support this diagnosis. It should be pointed out that in neither case were rosettes found in the material taken for biopsy from the femoral lesions. However, in both cases it was revealed at autopsy that the femoral tumor was a metastasis from a neuroblastoma, primary in an adrenal in one instance and in the left lumbar sympathetic chain in the other. In both cases rosettes were found only in the primary growth.

On the basis of these experiences, Willis [8] expressed great wariness

about a diagnosis of Ewing's sarcoma made on clinical (including roentgenographic) grounds alone. He cast doubt also upon the reliability of biopsy in this connection, and analyzed, largely to reject them, the findings in the relatively few cases published prior to 1940, which had been interpreted as Ewing's sarcoma proved by autopsy. His paper of 1940 bears careful reading for its evaluation of the reported autopsied cases of Ewing's sarcoma, even if it does appear that in some instances he has been over-critical in the standards he set.

There can be no doubt that Willis [8] was correct in holding that a presenting bone lesion which is in fact a metastasis of neuroblastoma may not be recognized as such on the basis of biopsy, and the following case from our own material illustrates this. The patient was a boy, 3 years of age, who was admitted because of pain in the left hip region and limping of 3 months' duration. A roentgenogram revealed a rarefying lesion in the neck of the left femur, resorptive destruction of the cortex in this area, and some periosteal new bone deposition on the cortex of the adjacent portion of the femoral shaft (Fig. 19). On the assumption that the lesion was a low-grade osteomyelitis, it was curetted, but this assumption could scarcely have been made if the child had been studied thoroughly before surgical intervention, for, on admission the child already presented a dilated left pupil, and evidences of general lymphadenopathy, especially prominent in the left cervical region. The tissue sections from the material curetted from the neck of the femur showed a malignant tumor. The tumor cells were supported in a connective tissue stroma which was loose in some places, rather collagenous in others, and tended to demarcate smaller or larger groups of the cells. The predominating type of cell was rather large and round, lacking a clear-cut cytoplasmic outline and having a large, pale, stippled nucleus. Although some smaller cells with dark hyperchromatic nuclei were present, a number of tumor giant cells, some of which had two or more nuclei, were also seen. The cytologic picture (Fig. 20) was not that which we associate with Ewing's sarcoma, nor, on the other hand, was it even vaguely suggestive of neuroblastoma. However, that the femoral lesion was in fact a metastatic neuroblastoma could be safely deduced from histologic examination of several enlarged lymph nodes from the left cervical region, which showed unmistakably the rosettes and other cytologic features of sympathetic neuroblastoma (Fig. 21). Unfortunately, we could not determine the site of origin for the neuroblastoma in this case, since there was no autopsy. The child died at home about 1 year after the onset of the complaints.

This case of sympathicoblastoma was peculiarly difficult to diagnose,

and makes us sympathetic to Willis'[8] contention that only carefully executed autopsies can prove or exclude neuroblastoma or completely justify a diagnosis of Ewing's sarcoma. However, that a large proportion of cases of sympathetic neuroblastoma have certain distinctive clinical and roentgenographic features which are useful in differentiating them from Ewing's sarcoma can be gathered from the mass of material on which Wyatt and Farber[25] have reported. As to our own 13 cases in which the diagnosis of Ewing's sarcoma was based on biopsy findings, it can be said that, in view of the uniformity of the cell type in these cases and its consistent resemblance to the cell type observed in the 4 autopsied cases, we feel reasonable confidence in assuming that we could not have been dealing in all 13 cases with metastases from neuroblastomas. This assumption seems all the more justified if one bears in mind that: None of the many tissue sections cut in these 13 cases showed the rosettes classic for neuroblastoma; if these 13 lesions represented metastases from neuroblastomas, the primary tumor in all these cases would have had to be silent and the cells in all the lesions would have had to be matured to, and only to, the sympathoblast level; all but one of these 13 patients was over 8 years of age, whereas the great majority of cases of neuroblastoma are seen in children under this age.

THE PROBLEM OF CARCINOMA AND OTHER MALIGNANT TUMORS WITH SKELETAL METASTASES IN RELATION TO EWING'S SARCOMA

The problem of the differential diagnosis of Ewing's sarcoma does not end with sympathicoblastoma, but may be raised also by metastatic carcinoma. That a solitary destructive bone lesion which is proved by biopsy to represent a metastasis may be the first clinical indication that the patient is suffering from carcinoma hardly needs to be stated. It is also common to find that, while the primary lesion is silent, the histologic picture of the neoplastic tissue in the biopsy specimen gives the clue to the site of the primary growth. Often, on the other hand, there is not sufficient cytologic differentiation to suggest the site of the primary lesion. Diagnostic difficulties arise in those cases in which the primary growth is silent and in which the neoplastic tissue in the metastatic focus is so undifferentiated as to present a more or less uniform pattern of round cells. Although this problem does sometimes arise in connection with biopsy diagnosis or even in connection with the evaluation of the autopsy findings in a suspected case of Ewing's sarcoma, it does not constitute a frequent or serious difficulty in the hands of an experienced pathologist. Still, Hirsch and Ryerson[26] pointed out that bronchial carcinomas (particularly small ones with

undifferentiated cells) may metastasize widely to the bones before being recognizable in the lung and thus raise problems of differential diagnosis from Ewing's sarcoma, a point of view also stressed by Sternberg.[27] Sternberg also cited a case in which a skeletal metastasis was regarded as Ewing's sarcoma, although he himself held that involvement of bone was secondary to an undifferentiated small-celled carcinoma of the breast.

Finally, it may not be amiss to point out that occasionally, in the course of evaluation of a biopsy specimen, one may have to make a differential diagnosis between Ewing's sarcoma on the one hand and Hodgkin's disease and lymphocytic lymphoma on the other. However, the latter conditions are so rarely primary in bones that one is not often confronted by this problem as a practical difficulty, and when they are not primary there the general clinical picture, in which involvement of lymph nodes occupies the foreground, helps to clarify the problem.

PROGNOSIS AND TREATMENT

With the exception of one patient, who was admitted to the hospital only 5 months ago and whose course is already downhill,* all of the patients in our series of 17 cases of Ewing's sarcoma have died. Some died within 6 months to a year, and all but 3 were dead within 3 years of the onset of the local clinical complaints. Of the 3 who survived longer, 2 lived for 3½ years and one for 5½ years after the onset of complaints. If calculated from the time of admission to the hospital, the period of survival is, of course, somewhat shorter in all cases. This doleful prognosis is also evident from the Memorial Hospital statistics recently cited by Coley,[28] who stated that of 71 cases there were none in which survival was beyond 5 years.

All of our patients received radiation therapy to the presenting bone lesion. However, it should be noted that these cases accumulated over the past 20 years, during which a good deal of progress has been made in radiation procedure, so that the results cannot be judged on a uniform basis. Also, in many of our patients, the presenting lesion was in a bone of the trunk, where the advantages of a large radiation dose are frequently more than counterbalanced by the danger of damage to internal organs. In addition, in those cases which ran a rapidly down-

---

* This patient died in August, 1946 (13 months after admission to the hospital) and autopsy amply confirmed the diagnosis of Ewing's sarcoma. Examination included a search for all possible primary sources of neuroblastoma. It further included the calvarium, practically all of the vertebral column (along with the sacrum), both innominate bones, the upper ends of both femora, part of a tibia, and many ribs. The marrow spaces of these bones were found extensively invaded by neoplastic tissue, which, where viable, showed the cellular characteristics of Ewing's sarcoma.

hill clinical course, it was almost certain that widespread metastases were already present at the time when radiation therapy was instituted and necessarily contributed to its ineffectiveness.

Inferences as to the influence of radiation therapy alone in the treatment of Ewing's sarcoma can best be drawn from those cases in which the disease is apparently still limited to a long bone. It is in these cases that cross-fire radiation of the whole affected bone from every possible angle is feasible, with a total tumor dose of as much as 4500 r. in one course of treatment, as advocated by Swenson.[5] Yet, in the 2 cases in our series which were adequately treated along these lines (one lesion in a fibula and the other in a humerus), and in which radiation therapy had a remarkable palliative local effect, the patients nevertheless succumbed to metastases 3½ and 5½ years, respectively, after the onset of the clinical complaints. Thus it would seem that radiation alone cannot be counted upon to produce a cure even in favorable cases. In such cases, radiation followed by surgery (amputation or disarticulation of the limb) may give better results, but our material includes no cases in which this combination has been tried. On the whole, we believe that even under the most favorable circumstances the ultimate prognosis in cases of Ewing's sarcoma is, as yet, very bad.

SUMMARY AND CONCLUSIONS

This study (based on 17 cases, 4 of which were autopsied) supports the existence, among the primary malignant tumors appearing in bones, of a tumor entity to which, because of Ewing's pioneer effort to single it out, the name of Ewing's sarcoma should be applied. Beyond the fact that it is a specific malignant tumor primary in bones, and that its cells show no osteogenic potentialities, there is still much to be learned in respect to its histogenesis. Study of the cytologic patterns in our material yields no support for Ewing's contention that the neoplastic cells are derived from capillary or vascular (or perivascular) endothelium. It is true that tumor areas which have become heavily invaded by blood vessels, especially in the wake of hemorrhage, often show tumor cells about capillary spaces or around larger vascular spaces in a so-called "perithelial arrangement," but perivascular orientation of the tumor cells is not a characteristic cytologic feature of this neoplasm. Also, when, in an occasional lesion, one finds formations in which cells show a ring-like arrangement (though not around a vessel), these formations can be seen to have resulted from degeneration of centrally located cells, the shadows of which are still perceptible. Such formations really have nothing in common with the rosette or pseudo-rosette formations of neuroblastoma.

We incline toward Oberling's idea that the tumor cells of Ewing's sarcoma are derived from the supporting framework (the reticular tissue) of the bone marrow, a framework which can be regarded as a mesenchymal or primitive form of connective tissue. Ewing described the type cell of the lesion as a small polyhedral cell with pale cytoplasm, a small, hyperchromatic nucleus, and a well defined cell border. However, as revealed in viable, well fixed and well stained neoplastic tissue, the type cell is actually found to have an ill defined cell border, little cytoplasm, and a fairly large, round or oval nucleus showing scattered chromatin. Nevertheless, to make a diagnosis of Ewing's sarcoma from a biopsy specimen, even if the latter is obtained by surgical incision, is sometimes difficult, because of secondary changes which the neoplastic tissue has undergone. Cell areas showing the characteristic structure are sometimes to be found only after many sections have been made and examined.

A diagnosis of Ewing's sarcoma on the basis of biopsy should not be made without giving consideration to the possibility that one may be dealing with a sympathetic neuroblastoma or anaplastic carcinoma metastatic to the affected bone. Such alternative possibilities as primary reticulum cell sarcoma of bone, Hodgkin's disease, malignant lymphoma, and even myeloma must be eliminated. If, in a patient suspected of having Ewing's sarcoma, enlarged lymph nodes are palpable (regionally to the affected bone, or elsewhere), these too should be examined anatomically, in consideration of alternative possibilities, since lymph nodes are not commonly involved in Ewing's sarcoma, at least in an early stage.

On the clinical side, in our cases of Ewing's sarcoma we found that the great majority of the patients were in the second decade of life. The clinical histories did not show trauma to be an instigating factor. In the majority of our cases, the presenting lesion was in a bone of the trunk. We found no evidence favoring the idea that the presenting bone lesion shows a characteristic, if not typical, roentgenographic picture of high diagnostic value.

Ewing's sarcoma has a most doleful prognosis, only one of the 17 patients in our series still being alive, and this one has been under our observation for only 5 months.* Fever, secondary anemia, and an increased sedimentation rate of the blood in a patient with Ewing's sarcoma are evidences that the course will be a fulminating one, ending in death within a few months. Radiation therapy alone, while often having a remarkable palliative local effect for some time, offers as yet but little hope so far as the ultimate issue is concerned. The combi-

41925.

nation of radiation therapy with surgery in favorable cases would seem to be more promising, but has not yet received sufficient trial to warrant a statement about its effects.

REFERENCES

1. Ewing, J. Diffuse endothelioma of bone. *Proc. New York Path. Soc.*, 1921, 21, 17–24.
2. Ewing, J. Further report on endothelial myeloma of bone. *Proc. New York Path. Soc.*, 1924, 24, 93–101.
3. Ewing, J. The Classification and Treatment of Bone Sarcoma. Report of the International Conference on Cancer, London. John Wright & Sons, Ltd., Bristol, 1928, pp. 365–376. (See: Endothelial Myeloma, p. 371.)
4. Neely, J. M., and Rogers, F. T. Roentgenological and pathological considerations of Ewing's tumor of bone. *Am. J. Roentgenol.*, 1940, 43, 204–210.
5. Swenson, P. C. The roentgenologic aspects of Ewing's tumor of bone marrow. *Am. J. Roentgenol.*, 1943, 50, 343–354.
6. Barden, R. P. The similarity of clinical and roentgen findings in children with Ewing's sarcoma (endothelial myeloma) and sympathetic neuroblastoma. *Am. J. Roentgenol.*, 1943, 50, 575–581.
7. Colville, H. C., and Willis, R. A. Neuroblastoma metastases in bones, with a criticism of Ewing's endothelioma. *Am. J. Path.*, 1933, 9, 421–429.
8. Willis, R. A. Metastatic neuroblastoma in bone presenting the Ewing syndrome, with a discussion of "Ewing's sarcoma." *Am. J. Path.*, 1940, 16, 317–331.
9. Oberling, C. Les réticulosarcomes et les réticulo-endothéliosarcomes de la moelle osseuse (sarcomes d'Ewing). *Bull. Assoc. franç. p. l'étude du cancer*, 1928, 17, 259–296.
10. Oberling, C., and Raileanu, C. Nouvelles recherches sur les réticulosarcomes de la moelle osseuse (sarcomes d'Ewing). *Bull. Assoc. franç. p. l'étude du cancer*, 1932, 21, 333–347.
11. Ewing, J. A review of the classification of bone tumors. *Surg., Gynec. & Obst.*, 1939, 68, 971–976. (See: Endothelioma, p. 975.)
12. Ewing, J. Neoplastic Diseases. A Treatise on Tumors. W. B. Saunders Co., Philadelphia, 1940, ed. 4, pp. 360–370.
13. Stout, A. P. A discussion of the pathology and histogenesis of Ewing's tumor of bone marrow. *Am. J. Roentgenol.*, 1943, 50, 334–342.
14. Parker, F., Jr., and Jackson, H., Jr. Primary reticulum cell sarcoma of bone. *Surg., Gynec. & Obst.*, 1939, 68, 45–53.
15. Connor, C. L. Endothelial myeloma, Ewing; report of 54 cases. *Arch. Surg.*, 1926, 12, 789–829.
16. Hamilton, J. F. Ewing's sarcoma (endothelial myeloma). *Arch. Surg.*, 1940, 41, 29–52.
17. Melnick, P. J. Histogenesis of Ewing's sarcoma of bone; with post-mortem report of a case. *Am. J. Cancer*, 1933, 19, 353–363.
18. Gharpure, V. V. Endothelial myeloma (Ewing's tumor of bone). *Am. J. Path.*, 1941, 17, 503–507.
19. Foote, F. W., Jr., and Anderson, H. R. Histogenesis of Ewing's tumor. *Am. J. Path.*, 1941, 17, 497–502.
20. Edwards, J. E. Primary reticulum cell sarcoma of the spine. Report of a case with autopsy. *Am. J. Path.*, 1940, 16, 835–844.
21. Gall, E. A., and Mallory, T. B. Malignant lymphoma; a clinicopathologic survey of 618 cases. *Am. J. Path.*, 1942, 18, 381–429.
22. Hutchison, R. On suprarenal sarcoma in children with metastases in the skull. *Quart. J. Med.*, 1907–08, 1, 33–38.

23. Tileston, W., and Wolbach, S. B. Primary tumors of the adrenal gland in children. Report of a case of simultaneous sarcoma of the adrenal gland and of the cranium, with exophthalmos. *Am. J. M. Sc.*, 1908, 135, 871–889.

24. Wright, J. H. Neurocytoma or neuroblastoma, a kind of tumor not generally recognized. *J. Exper. Med.*, 1910, 12, 556–561.

25. Wyatt, G. M., and Farber, S. Neuroblastoma sympatheticum; roentgenological appearances and radiation treatment. *Am. J. Roentgenol.*, 1941, 46, 485–495.

26. Hirsch, E. F., and Ryerson, E. W. Metastases of the bone in primary carcinoma of the lung; a review of so-called endotheliomas of the bones. *Arch. Surg.*, 1928, 16, 1–30.

27. Sternberg, C. Zur Frage des sogenannten Ewings tumor. *Frankfurt. Ztschr. f. Path.*, 1935, 48, 525–532.

28. Coley, B. L. Tumors of Bones and Joints. In: Bancroft, F. W., and Murray, C. R. (eds.) Surgical Treatment of the Motor-Skeletal System. J. B. Lippincott Co., Philadelphia, 1945, p. 349.

DESCRIPTION OF PLATES

PLATE 8

FIG. 1. Roentgenograph showing rarefaction of a medial femoral condyle and of the cortex of the shaft just above the condyle. There is also an indistinct, small, soft tissue mass overlying the cortex above the condyle, the faint radiopacity representing periosteal new bone apposition. Although the picture suggests that the lesion might be a malignant tumor, there is nothing in it to justify the specific conclusion that it represents Ewing's sarcoma. The limb was disarticulated a week later, and Figure 9, showing the femur in coronal section, reveals how much greater was the actual involvement than was apparent roentgenographically. The patient, a male of 19 years, died 5 months after admission to the hospital, and an autopsy was done.

FIG. 2. Roentgenograph showing irregular rarefaction of the neck of a femur. The cortex is relatively unmodified and there appears to be no soft tissue mass overlying it. It would hardly be suspected from this picture that the lesion was a neoplasm. On a clinical basis, it was thought to be tuberculosis. Surgical intervention undertaken on this premise revealed Ewing's sarcoma. The patient, a boy of 14 years, died at another hospital 4 months after admission to our hospital, and no autopsy was done.

FIG. 3. Roentgenograph showing rarefaction and disruption of the lateral cortical outline of an iliac bone—a picture pointing clearly to the presence of a malignant tumor, although not necessarily Ewing's sarcoma. This was the picture of the presenting bone lesion on admission, and, although local clinical complaints were of only several days' standing, a tumor mass of the size of a neonate head could already be felt attached to the iliac fossa and the lateral aspect of the ilium. Pulmonary metastases were already present. The patient, a girl of 16 years, died 4 months after admission to the hospital, and an autopsy was done. Figure 11 shows the removed affected innominate bone in longitudinal section through the region of the acetabulum.

FIG. 4. Roentgenograph showing an extensive, destructive, rarefying lesion, involving the body and ascending ramus of a pubic bone and definitely suggesting a malignant neoplasm. This was the picture of the presenting bone lesion on admission, and the clinical complaints referable to the ipsilateral hip joint were already of 8 months' duration. At this time, lesions were evident in some other bones also and in the lungs. The patient, a girl of 17 years, died at another hospital about 8 months after admission to our hospital, and no autopsy was done.
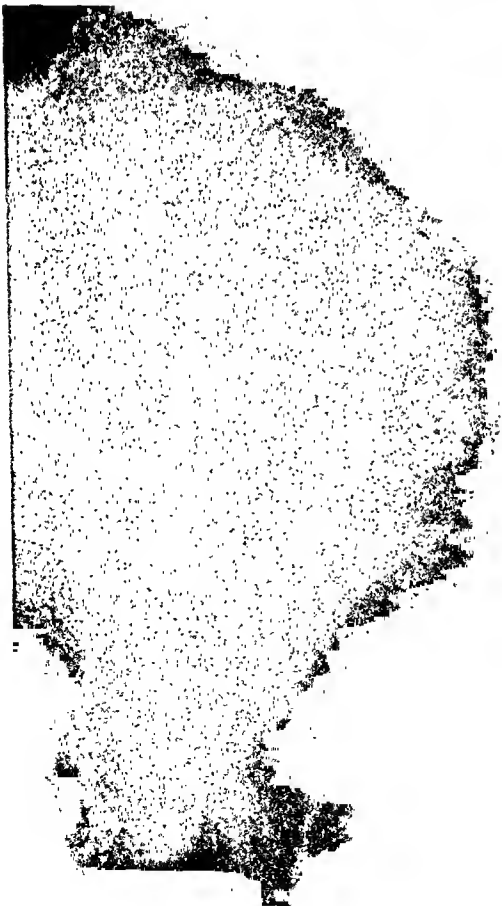
Plate 8

Lichtenstein and Jaffe

Ewing's Sarcoma of Bone

PLATE 9

FIG. 5. Roentgenogram showing extensive mottled rarefaction of the upper half of a tibia without any significant deposition of new bone by the periosteum. Although the picture of this presenting lesion could suggest a malignant tumor. there is nothing in it to justify, by itself, the conclusion that the lesion represents Ewing's sarcoma. Clinically it was suspected that it had an inflammatory basis. However, it was a Ewing's sarcoma and the patient, a girl of 18 years. died 11 months after admission to the hospital. An autopsy was done at Montefiore Hospital. -

FIG. 6. Roentgenograph showing extensive destructive rarefaction of the shaft of a humerus, associated with onion-skin-like periosteal new bone deposition in the lateral side and a thick mass of neoplastic tissue overlying the bone on its medial side. This mass, in its upper region, presented some transverse radio-pacities. The picture is clearly that of a malignant tumor, but if one had to be guided by the roentgenograph alone one would say that the lesion was an "osteolytic" osteogenic sarcoma rather than a Ewing's sarcoma. The patient was a girl, 8 years old, who died at home 7 months after admission to the hospital, and no autopsy was obtained.

FIG. 7. Roentgenograph of the upper portion of the shaft of a humerus. The cortex is not thickened but presents an irregular, moth-eaten appearance, while the soft tissue tumor mass overlying it shows more or less transverse streaks of radiopacity. This picture. too. suggests an osteogenic rather than a Ewing's sarcoma. The patient was a male. 24 years of age, who died at home 5½ years after admission to the hospital. and no autopsy was obtained. (See also Fig. 8.)

FIG. 8. Punched-out rarefactions in the calvarium of the patient referred to in the legend for Figure 7, representing lytic destruction of the bone by neoplastic tissue. This was the appearance of the calvarium 6 months before the patient died. It is a nondescript appearance which could have been created also by plasmatocytic myeloma or metastatic carcinoma.

PLATE 9

Lichtenstein and Jaffe

Ewing's Sarcoma of Bone
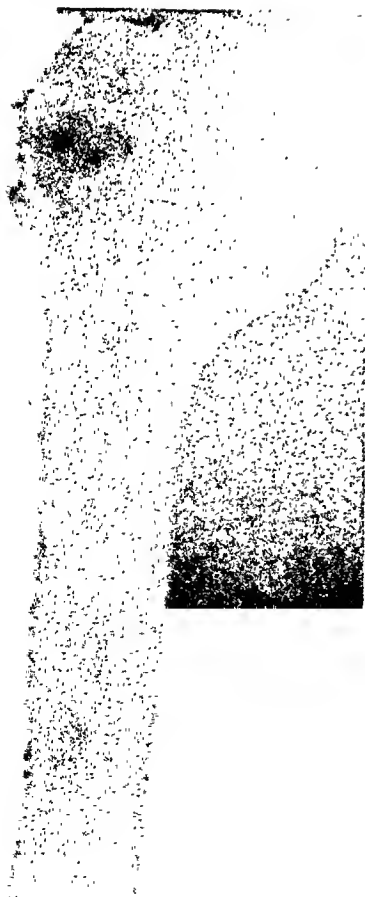
PLATE 10

Fig. 9. Photograph showing sagittally cut surface of the femur of which Figure 1 is the preoperative clinical roentgenograph. This picture shows clearly that the extent of tumorous involvement of the bone is far greater than could have been suspected from the preoperative roentgenograph. Of note are the nodules of neoplastic tissue at the upper end of the major marrow cavity.

Fig. 10. Roentgenograph of a thin slice of the bone, including the lower end. cut in the sagittal plane from the femur illustrated in Figure 9. Even here the actual extent of the involvement is not reflected roentgenographically.

Fig. 11. Photograph of the cut surface of the affected innominate bone. and the neoplastic tissue adherent to it, from the case illustrated in Figure 3. The bone is cut in the longitudinal plane, through the region of the iliac fossa. The iliac portion of the bone has been largely destroyed and replaced by a tumor mass visible to the left. while on the right is the tumor mass which extended into the pelvis.

Fig. 12. Photograph showing extensive tumorous involvement of part of the vertebral column in the case also illustrated in Figures 1. 9. and 10.
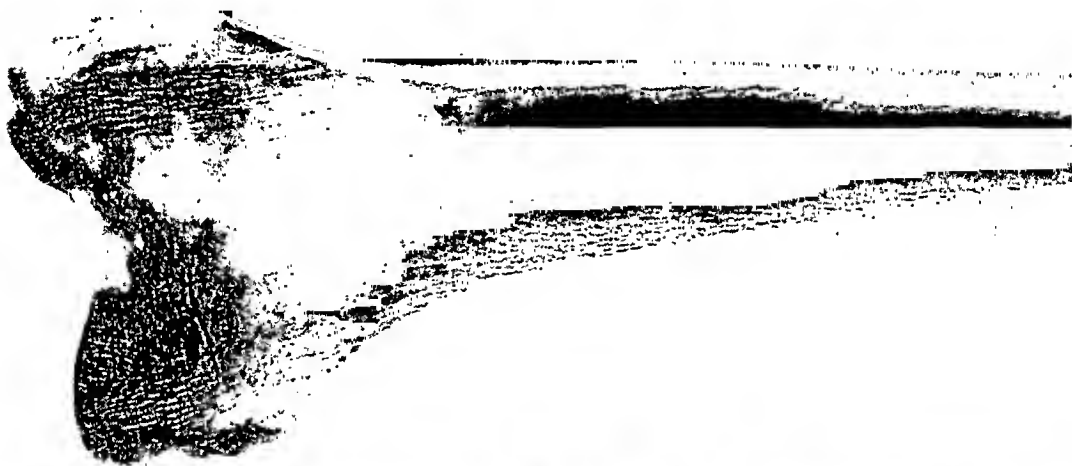
PLATE 11

FIG. 13. Photomicrograph showing the general cytologic pattern in a cellular and relatively unmodified area of a Ewing's sarcoma. Neoplastic tissue is poor in blood vessels and the nuclei are enmeshed in a loose and more or less vacuolated cytoplasmic fabric. The tissue came from the case illustrated also in Figure 7. × 500.

FIG. 14. Photomicrograph showing the general cytologic pattern in a cellular and relatively unmodified area from a Ewing's sarcoma in another case. In this lesion, the nuclei are crowded together and the cell boundaries are not distinct. The tissue came from a male, 18 years old, in whom the presenting lesion was in a fibula and who died 3½ years after the onset of his complaints. × 500.

FIG. 15. Photomicrograph showing the cytologic pattern presented by most of the neoplastic tissue removed for biopsy, in the case illustrated also in Figure 4. From the tissue illustrated, in which small, dark, hyperchromatic nuclei are intermingled with leukocytes, it would not have been possible to tell that one was dealing with a tumor, much less a Ewing's sarcoma. It was only after many sections had been prepared from all the tissue submitted that a few microscopic fields of well preserved neoplastic tissue were found, permitting a diagnosis. × 250.

FIG. 16. Photomicrograph showing the cytologic picture presented by fields of grossly hemorrhagic neoplastic tissue in a case of Ewing's sarcoma in a male of 19 years, whose presenting lesion was in an iliac bone. It was only in the areas in which the neoplastic tissue was hemorrhagic and necrotic that one noted the pattern of tumor cells collaring capillary or larger vascular spaces. The tumor cells themselves do not make up the walls of the vascular spaces. × 250.

Lichtenstein and Jaffe   Ewing's Sarcoma of Bone
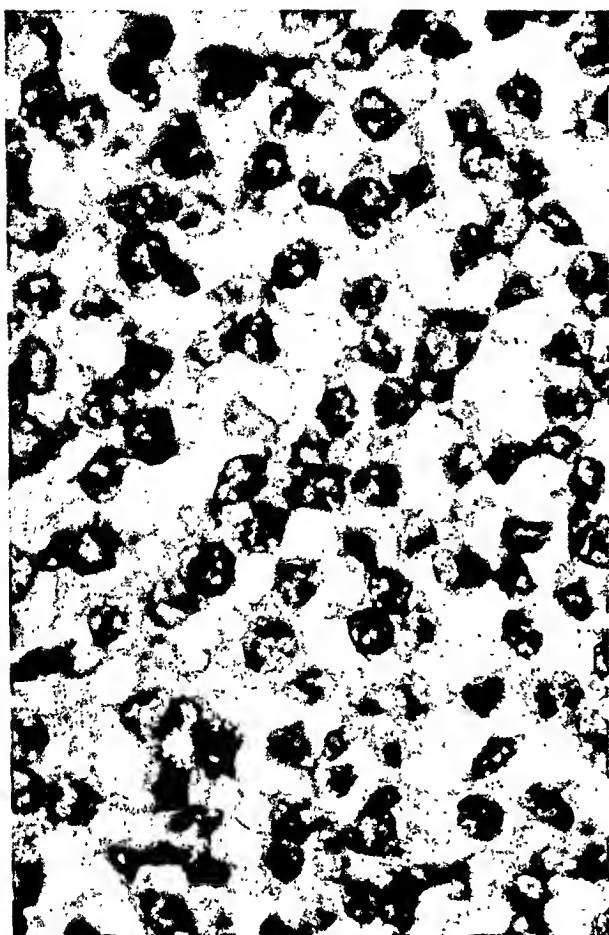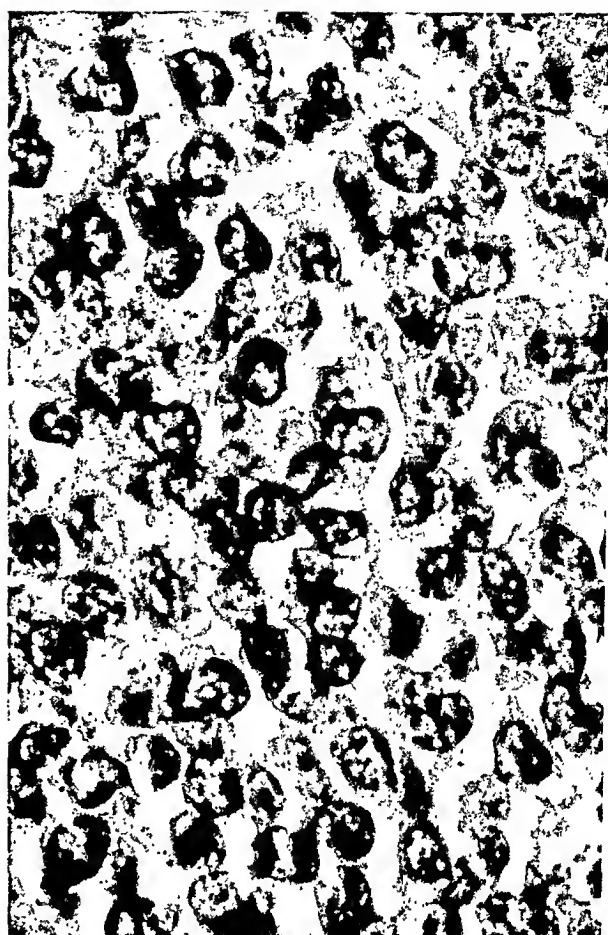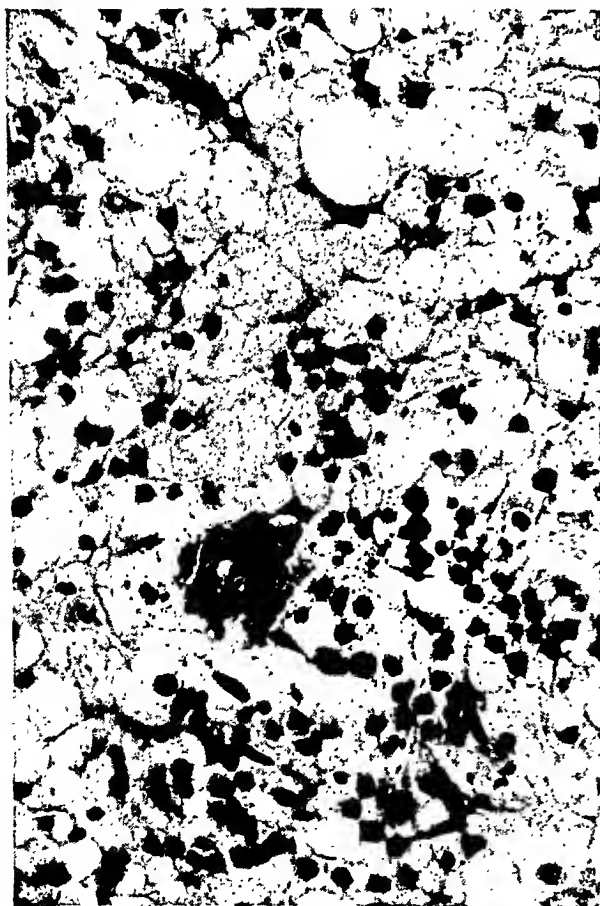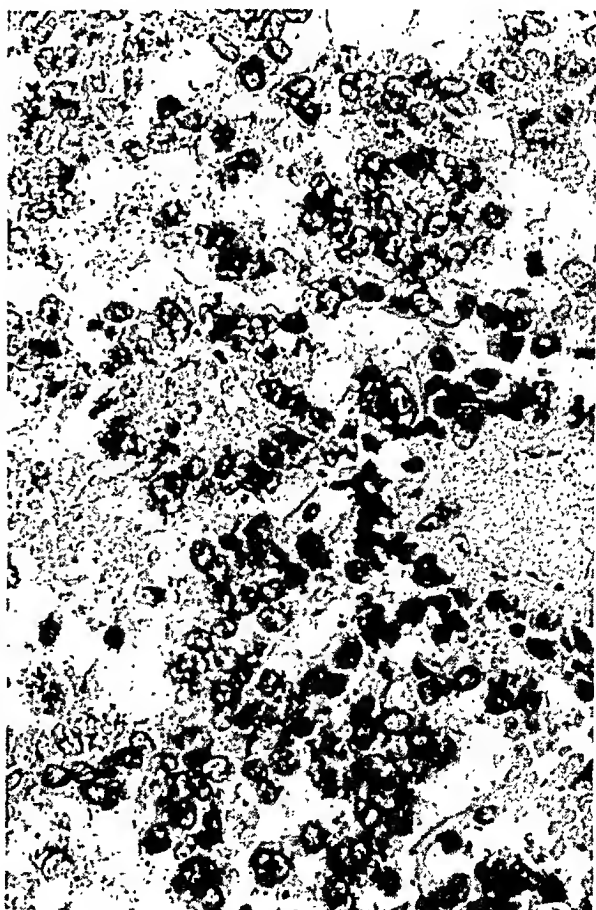
PLATE 12

FIG. 17. Photomicrograph showing the general cytologic pattern of a tumor field in a metastatic lesion of Ewing's sarcoma in a rib in the case illustrated also in Figures 1. 9. and 12. As a result of cell degeneration in some places. there are suggestions of ring-like formations. (For comparison with Fig. 18.) × 500.

FIG. 18. Photomicrograph showing the general cytologic pattern of a Ewing's sarcoma in a fibula of a girl. 4 years of age. The "rosette-like" formations are constituted by rings of viable tumor cells surrounding cores of degenerated tumor cells, the shadows of which are still perceptible. Such spurious rosettes or pseudorosettes may be compared with the genuine ones shown in Figure 21. × 250.

FIG. 19. Roentgenograph showing a rarefying. destructive lesion in the neck of a femur, a lesion which was clinically considered to have an inflammatory basis. One could not tell from this picture that the lesion was actually a metastatic neuroblastoma. The patient was a boy of 3 years whose clinical complaints were pain in the involved hip region and limping of 3 months' duration.

FIG. 20. Photomicrograph showing the general cytologic pattern of the neoplastic tissue curetted from the femoral neck in the case illustrated in Figure 19. No more definitive diagnosis could be made from the curettings than that we were dealing with a malignant tumor. × 500.

FIG. 21. Photomicrograph showing the general cytologic pattern of an involved lymph node removed from the cervical region in the case illustrated in Figures 19 and 20. Of note are the classic rosettes, permitting a definitive diagnosis of sympathicoblastoma in this case. × 500.

Lichtenstein and Jaffe   Ewing's Sarcoma of Bone
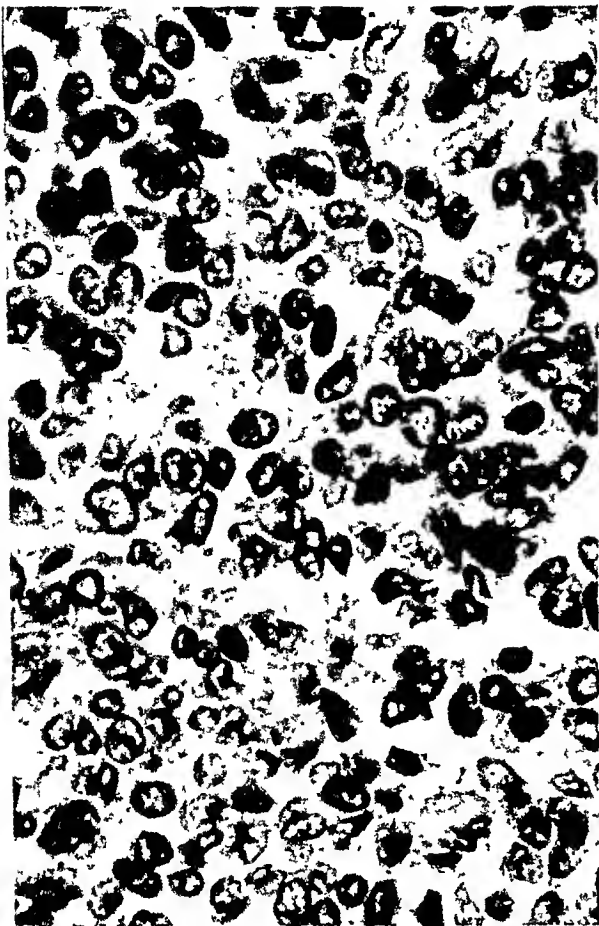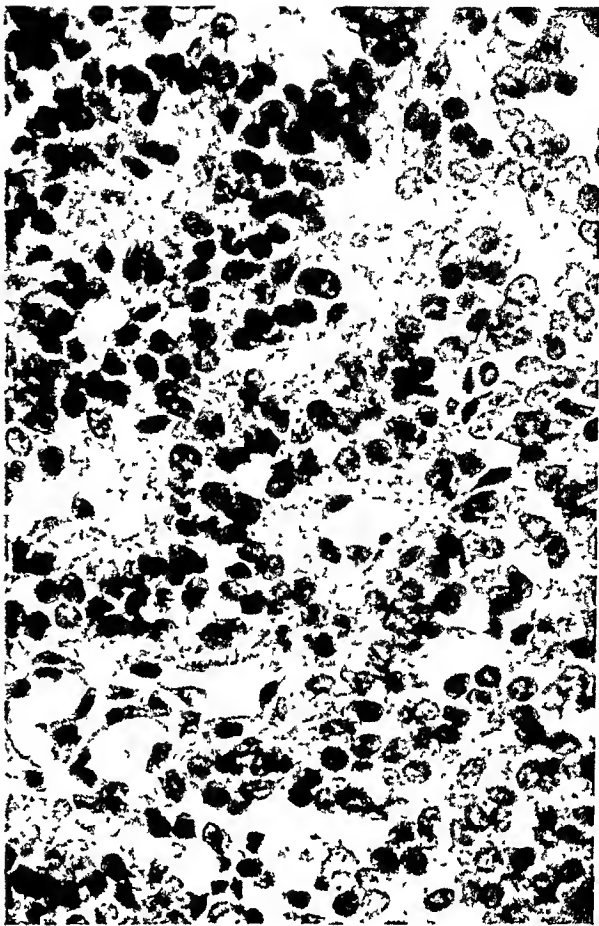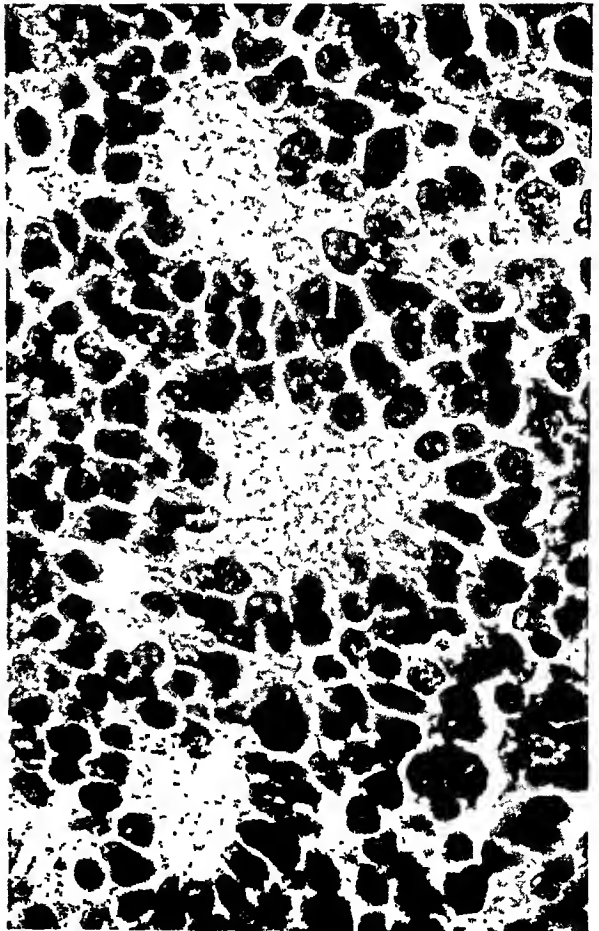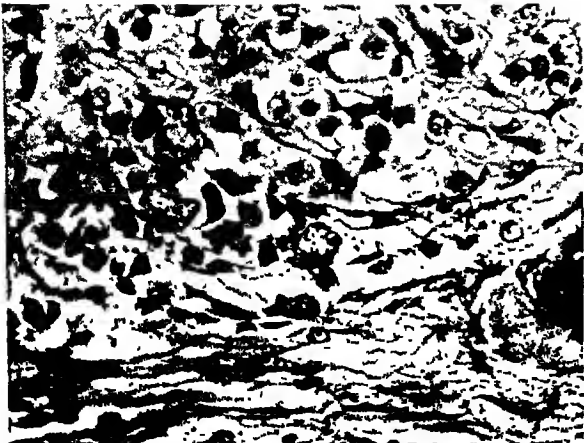
FATTY INFILTRATION OF THE LIVER IN PATIENTS WITH MONGOLISM AND IN CHILDREN WITH HYDROCEPHALY AND MICROCEPHALY *

EDWARD C. ROOSEN-RUNGE, M.D.

(From the Wallace Research Laboratory, Wrentham State School, Wrentham, Mass.)

The present investigation was undertaken in connection with a comprehensive study on mongolism carried out by Dr. Clemens E. Benda and his collaborators at the Wallace Research Laboratory of the Wrentham State School.

The pathology of the mongoloid liver has not been described before. In Brousseau's [1] monograph on mongolism in 1928, the liver is not mentioned.

MATERIAL AND METHODS

Forty-three livers of mongoloid patients were examined microscopically. Thirty-four of these came from cases which were studied clinically during life and of which extensive autopsy reports existed. The diagnosis was made on the grounds of a thorough examination of the many stigmata of mongolism. The endocrine glands of most cases were examined by Dr. Benda and his results are published elsewhere.[2]

Almost all of the material was fixed in 10 per cent formalin, which was neutralized in about half of the cases. Unfortunately, very little material was available for glycogen stains. The examination for glycogen appears very desirable and will be carried out in the future. Frozen and paraffin sections were made of almost all of the livers and the following staining methods were employed: hematoxylin and eosin, sudan IV for fat, Heidenhain's azocarmine-aniline blue or Mallory's triple stain, and Giemsa's stain in a few cases.

The livers of the mongoloid patients were compared with a control series of 49 livers of mentally deficient patients of 1 to 30 years of age (Table III). The control series comprised 35 idiots and 14 imbeciles and morons with varying etiologic factors: developmental and hereditary disorders, birth injuries, post-infectional and post-traumatic states, as well as a few of undetermined origin. The cases were taken from the same group of 170 autopsies of which the mongoloid patients were another part. All those cases were used which were of comparable age and of which sufficient material was available.

RESULTS

The livers of mongoloid patients tend to be small (see Tables I and II). Of 34 livers, 20 were underweight, 8 within normal range, and 6 overweight. Extreme underweight (below 25 per cent) was found in

10 cases (C 94, C 181, 15, 152, 17, 139, 58, 126, 83, and 71), extreme overweight in 3 cases (C 62, 148, and 65). While the average hepatic weight is considerably below normal standards, it is rather above the average for mentally deficient patients other than mongoloids, as can be seen in Table I.

A connection between the fat content of the livers and their weight cannot be established. Of the 3 livers with a great increase in weight, one had an extreme amount of fat and 2 showed a moderate amount. Of 10 extremely light livers, 5 had a marked degree of fatty deposit, one had a moderate amount, and 4 showed hardly any fat.

Many livers showed a yellowish tinge and on section revealed a light surface, which made one suspect fatty deposits. Usually the micro-

TABLE I
Weights of Livers *

	Mongoloids, aged 9 days to 28 years		Mentally deficient patients, exclusive of mongoloids, aged 1 to 30 years	
No. of cases	34		49	
Cases normal or ± 5% in weight	8	23.5%	6	12.2%
Cases, overweight	6	17.7%	7	14.3%
Cases, underweight	20	58.8%	36	73.5%
Cases of extreme underweight, below 25%	10	29.4%	20	40.8%
Cases below 50%	0	0	5	10.2%

* For standard values the table of Coppoletta and Wolbach was used (Coppoletta, J. M., and Wolbach, S. B. Body length and organ weight of infants and children. *Am. J. Path.*, 1933, 9, 55–70.)

scopic examination revealed far more fat to be present than was estimated at autopsy. Only one liver (case 148) was found to be actually oozing fat and this liver was also the only one that floated on water. All livers showed a smooth surface. Sometimes increased lobular markings were seen, suggestive of an increase in fibrous tissue. In general the macroscopic appearance of the livers of mongoloid patients is not characteristic.

The main pathologic features are presented in condensed form in Table II. The two outstanding points are: the clear division into two age groups, above and below 2 years, which differ in their essential features; the great frequency of pathologic amounts of fat in the older age group, often accompanied by a moderate increase in fibrous tissue.

Fatty Vacuolization

Twenty-five of the 31 livers from patients more than 2 years of age contained a distinctly abnormal amount of fat. The character of these fatty deposits was clearly recognized in the most severe cases (++++),

TABLE II

Condensed Chart of the Findings in 43 Livers of Mongoloid Patients

Case no.	Sex	Age	Weight in gm.	Fat	Fibrosis	Congestion	Tuberculosis of liver†	Cause of death
*C 76	F	9 days	90	–	–	+++	–	?
**C 94	F	6 wks.	76	–	–	++	–	?
*C 162	M	2 mos.	?	–	–	+++	–	?
*C 181	M	2½ mos.	75	–	+	++	–	?
*C 95	M	4½ mos.	?	–	–	+++	–	?
*C 62	F	6 mos.	260	+++	+	–	–	Lobar pneumonia
120	M	7 mos.	240	+	++	++++	–	Multiple abscesses
15	M	7½ mos.	160	–	–	++++	–	Scarlet fever, bronchopneumonia
152	M	8 mos.	190	+++	+++	+	–	Meningitis
154	M	1 6/12 yrs.	290	++	++	++	–	Bronchopneumonia
140	M	1 8/12 yrs.	?	–	–	+	+	Bronchopneumonia
102	M	1 10/12 yrs.	390	+++	+++	++	–	Tuberculous meningitis
*C 102	F	2 4/12 yrs.	?	+++	+++	++	–	?
99	M	4 5/12 yrs.	570	+++	++++	+++	–	Brain edema
57	F	4 5/12 yrs.	450	+++	+++	–	–	Septic sinus thrombosis
103	M	6 7/12 yrs.	680	+++	++	++	–	Lung abscess
124	M	7 yrs.	640	+++	+	++	+	Lung embolism
118	M	7 3/12 yrs.	605	++	+++	++	–	Tb. of lungs
47	F	8 7/12 yrs.	800	+++	+++	++	–	Diphtheria, pneumonia
25	M	8 8/12 yrs.	?	+	+	–	+	Bronchopneumonia
125	M	8 9/12 yrs.	670	+++	+++	++	–	Tb. of lungs
134	F	9 7/12 yrs.	710	+++	++	++	+	Intussusception
17	M	9 8/12 yrs.	460	+++	+++	+	–	Bronchopneumonia
141	M	10 4/12 yrs.	740	+++	+++	++	+	Tb. of lungs
139	F	11 1/12 yrs.	690	+++	++	+	–	Bronchopneumonia
133	F	12 yrs.	?	+++	–	–	–	Volvulus
82	F	13 10/12 yrs.	890	+++	+	–	++	Tb. of lungs
155	M	14 4/12 yrs.	?	+++	+	–	+	Tb. of lungs
32	M	14 11/12 yrs.	830	++	+++	–	–	Bronchopneumonia
116	F	15 4/12 yrs.	1100	+++	–	–	–	Tb. (miliary)
100	F	15 8/12 yrs.	1135	+++	+++	+	++	Tb. of lungs
148	M	15 10/12 yrs.	1650	+++	+++	–	–	Tb. of lungs
85	M	16 yrs.	1400	++	+	++	+	Status epilepticus
58	M	16 3/12 yrs.	880	+++	+++	++	++	Tb. of lungs
81	M	17 yrs.	1140	++	+++	+	–	Tb. of lungs
90	M	17 yrs.	1590	–	–	–	–	Gangrene of lung
126	F	17 1/12 yrs.	1050	+++	+++	++	–	Tb. of lungs
83	M	17 9/12 yrs.	880	++	+++	+	+	Tb. of lungs
65	M	18 1/12 yrs.	2100	++	+++	–	–	Tb. of lungs
146	M	18 4/12 yrs.	1510	+++	+++	–	–	Tb. of lungs
*M 3	F	20 yrs.	?	+	+	+++	+	Tb.
22	M	20 6/12 yrs.	?	++	+++	+++	–	Bronchopneumonia
71	F	28 5/12 yrs.	820	+++	–	++	+	Tb. of lungs

+ = Slight degree, probably not pathological.
++ = Definitely pathological, but not pronounced.
+++ = Marked degree.

* Material from these cases was contributed by other hospitals.
† The presence of tuberculous foci is marked with a cross regardless of the degree of involvement.

in which all liver cells were filled with fat droplets up to 50 μ in diameter (Figs. 2 and 3). These extreme cases still showed the lobular pattern. The liver cords radiating from the central veins were not disrupted. There was, in addition, a considerable amount of cytoplasm between the fat droplets, which showed no degenerative signs. The impression was that of fatty "infiltration" rather than of "degeneration," and this also held true for most of the less extreme cases. Typ-

TABLE III

Comparative Pathology of the Liver

	Mongoloids, aged 1 to 28 years		Mentally deficient patients, exclusive of mongoloids, aged 1 to 30 years	
No. of cases	34		49	
Cases with tuberculosis	17	50.0%	19	38.8%
Cases without fat or with small amounts of fat (+ or −)	8	23.5%	29	59.2%
Cases with fat (++ to ++++)	26	76.5%	19	38.8%
Cases with fat, with tuberculosis	13	76.5% of cases with tuberculosis	8	42.1%
Cases with fat, without tuberculosis	13	76.5% of cases without tuberculosis	11	36.7%
Cases with much fat (+++ to ++++)	17	50.0%	10	20.4%
Cases with much fat, with tuberculosis	8	47.1% of cases with tuberculosis	3	15.8%
Cases with much fat, without tuberculosis	9	52.9% of cases without tuberculosis	7	23.3%
Cases with fibrosis (++ to ++++)	19	55.9%	6	12.3%

ically, the fat was accumulated around the portal vessels and little was found near the central veins (Fig. 1), but 5 cases definitely showed the opposite pattern—a distinct pericentral accumulation. In 2 instances there was a considerable increase in fat droplets around the central veins as well as around the portal vessels, leaving the intermediate zones of lobules free from fat. In the extreme cases, the fat filled the liver completely and uniformly. In one case (no. 140) the Kupffer cells were almost all filled with fat in very fine granules in addition to a moderately severe fat deposit in the liver cells which showed no other unusual features (Fig. 7).

A remarkable distribution of fat was seen in case 124 in which all arteries and arterioles were very thick-walled and partly occluded. The parenchyma was filled quite uniformly with fatty vacuoles of

varying sizes, but the liver cells directly adjacent to the affected vessels contained very little fat. The vessels appeared lined on the outside by one layer of almost normal liver cells (Fig. 8).

Of 25 nonmongoloid mentally deficient children under 15 years of age, 11 showed fat in the liver and in 8 the pathologic picture was not essentially different from that found in mongolism. In all there was hydrocephalus or microcephalus with definite signs of brain pressure. A description and discussion of the 5 most extreme cases will be given later in this paper.

Fibrosis

An increase in the fibrous tissue occurred less frequently than did fatty vacuolation (18 of 31 cases over 2 years of age). The increase was always predominantly periportal, even if the fat was accumulated pericentrally. The outstanding fact is that the proliferation usually remained moderate in degree and showed very little tendency to progress into the lobules at the expense of the parenchyma. Nodular cirrhosis was never found. Typically, the capsule was thickened to about twice its normal size, but was even and smooth, and the periportal tissue appeared increased with thickened septa dividing the lobules. Only one liver (no. 65) showed a very high degree of fibrosis but even its surface was smooth and replacement of liver cells by fibrous tissue was moderate. Thirteen of 26 cases above 7 years of age showed no other abnormalities but fatty deposits and some fibrosis.

Degenerative Phenomena and Congestion

At least half of the cases presented degenerative phenomena of varying degrees in the liver cells. In most cases the degeneration was not uniform and consisted only of swelling of the cells. Necrosis and nuclear degeneration were infrequent. When they occurred, big fat vacuoles were often seen in the affected cells (Fig. 4). One case (no. 125) showed a high degree of necrosis throughout the liver without any indication of regenerative activity. This liver was not typical of mongolism, as there was no fat detectable in the cells. The patient was a Negro, and some doubt is thrown on the diagnosis through the fact that his pituitary and thyroid glands were also not typical of mongolism.

Congestion is found in connection with so many diseases and is so often connected with terminal circulatory failure, either local or general, that it was not surprising to find it in almost half of the cases. It was almost always confined to those areas which did not show fatty vacuolation (Fig. 5), but in 2 cases the opposite distribution was observed.

Congestion was a remarkable feature in some cases of the age group

below 2 years. Nine of the 12 livers in this group showed a considerable amount, and 3 showed engorgement of a severe degree accompanied by pericentral hemorrhagic necrosis. In these cases the liver cords were greatly compressed by the engorged sinusoids (Fig. 6).

In general, it appears that degenerative phenomena and congestion occur secondarily in livers which are primarily fatty. The obviously abnormal metabolism probably predisposes the liver tissue to subsequent disease. The existence of cases which show extreme amounts of fat without apparent degeneration makes it probable that the variation in the degenerative signs observed was due to the action of infectious diseases, chiefly tuberculosis, on the abnormal liver.

Among the 31 livers of mongoloid patients who were 2 years or more of age, there was one which was essentially normal (no. 90). It is interesting to note that this case was regarded clinically as "borderline." The weight of the brain was the only normal weight in the series and the gonads were better developed than is usual in a mongoloid patient.

DISCUSSION

Fatty livers occur most frequently in chronic alcoholism, in tuberculosis, and in general obesity. The last two of these conditions must be considered here. Half of the mongoloid patients died of tuberculosis and the liver was involved in 76.5 per cent of these cases. But a closer study of Tables II and III reveals that the fatty deposits occurred without relation to the presence or absence of tuberculosis. In the first place, fat occurred with exactly the same frequency, 76.5 per cent, in those cases which had no tuberculosis. In Table II this is most easily seen when attention is paid to the age group between 2 and 10 years, in which tuberculosis as the cause of death occurred only twice in 11 cases. However, 8 cases showed abnormal amounts of fat in the liver. In one of the 2 tuberculous patients no fat was found.

Furthermore, a comparison of the figures in Table III demonstrates the same fact in the control series: the incidence of fatty livers in cases with tuberculosis was not different from the incidence in the series as a whole, including the cases without tuberculosis. Tuberculosis as a cause of death was slightly less frequent (38.8 per cent) than in the mongoloid patients (50 per cent). Fat in the livers occurred only half as frequently (38.8 per cent compared with 76.5 per cent), but the incidence in cases with tuberculosis (42.1 per cent) differed little from that in cases without tuberculosis (36.7 per cent). It can, therefore, be concluded that the rôle which tuberculosis plays in producing fatty livers in the mongoloid patient can only be slight and that some other explanation must be found for the phenomenon. The fact that the

same is true for mentally deficient patients other than mongoloids actually raises some doubt as to the mechanism involved in the origin of fatty livers in tuberculous cases. It appears desirable to reinvestigate this problem in a large series of material.

Whether the fatty livers in mongoloid patients may be due to general obesity is a more difficult problem. Obesity as such is not an etiologic entity. It may arise through a predominantly fatty diet as well as through a variety of metabolic disorders. The patients investigated in this paper all received a well balanced diet. The obesity of the individuals varied a great deal and no connection could be found between the degree of obesity and the fattiness of the liver. Many patients with extremely fatty livers were not at all obese. However, the average relative weight of the mongoloid is high from the third year of life on (for tables on the weight and length of mongoloid children see Benda [3]). The mongoloid infant is underweight, often very much so; the gradual change to overweight takes place during the second year of life. Fat appears in the livers at about the same time. Both events seem to be consequences of a metabolic disorder.

When dogs are deprived of both the pituitary and thyroid glands, the liver shows a picture very similar to that found in the mongoloid patient.[4] Extensive fatty deposits are seen, often followed by periportal fibrosis. When the thyroid alone is removed, the fatty changes are far less pronounced. Hypophysectomy alone produces a picture of hepatic cirrhosis in dogs. In many cases this is accompanied by fatty deposits (Graef, Negrin, and Page [5]). These authors believe that the infundibulum and the stalk, rather than the pituitary gland, exert an influence on the fat metabolism of the liver. The picture which they described resembles that of the liver of mongoloids in some respects, but the analogy is not nearly as close as in the dogs which were deprived of both the pituitary and thyroid glands. In particular, the great amount of fibrosis leading to a typical nodular cirrhosis with atrophy of liver cords was never observed in mongoloids. It is, of course, questionable whether the dog and man react alike to a deficiency of their endocrine glands. Results seem to point to a greater tendency of the dog's liver to react with severe proliferation of fibrous tissue. Dogs subjected to prolonged feeding of a high-fat diet first showed fat deposits in the livers; later a typical cirrhosis developed.[6] In the mongoloid, fatty infiltration is the rule and fibrosis often follows, but reaches only a moderate degree, while the fat may increase to extreme amounts.

Graef, Negrin, and Page [5] have reported 2 cases of tumor indirectly involving the pars nervosa and the stalk of the pituitary gland, and

showing considerable fatty deposit in the liver with "otherwise normal" lobules. These findings seem to point in the same direction, namely, that in man, deficiency of the pituitary body or its stalk does not cause cirrhosis but predominantly causes a fatty change in the liver. Whether brain pressure plays a part in the mongoloid patient in affecting the pituitary body and hypothalamus, as described by Kraus [7] in certain tumors causing intracranial pressure, is a point of speculation. Morgan [8] has demonstrated defects in the hypothalamus of mongoloids, and intracranial pressure—not spinal fluid pressure—probably exists at some time during the life of the patient with mongolism.[9]

In this connection, it is an interesting fact that there are a number of cases in the control series of mentally deficient patients in which a fatty liver might also be attributed to brain pressure and a damaged infundibulum.

Case 97. 1-year-old male, idiot, microcephalus, died of bronchopneumonia. Not obese. Large old intracranial hemorrhages. Cortex atrophic; weight of brain, 138 gm. Skull small, 12 inches in circumference, sutures firmly united. The liver showed considerable fatty infiltration (+++).

Case 73. 3½-year-old male, imbecile, hydrocephalus of 35 inches in circumference, which was the cause of death. 8,000 cc. of spinal fluid was removed. At autopsy, body in fair state of nutrition. The liver showed extreme amounts of fat (++++).

Case 40. 5-year-old female, idiot, microcephalus. Cause of death: status epilepticus. Small and thin child. Blind. Head circumference, 17¼ inches. Hydrocephalus internus. Widened infundibulum. Liver underweight, fatty (+++).

Case 98. 13-year-old female, idiot, birth injury. Very obese. Cause of death: cystic brain. Skull thickened. Brain weight, 498 gm. Cystic degeneration of right and left occipital, and right parietal and temporal lobes. Liver weight, 820 gm.; much fatty infiltration (+++).

Case 64. 14-year-old female, idiot, hydrocephalus. Cause of death: status epilepticus. Well nourished. Brain weight, 505 gm. Extreme internal hydrocephalus, infundibulum enlarged to an opening of 3 cm. depth and 1.5 cm. width. Liver weight, 640 gm. Severe fatty infiltration (+++).

In all of these 5 cases the cause of the fatty infiltration in the liver was not obvious, but it appears remarkable that they should all have brain damage of a kind which produces brain pressure. In case 40 and case 64, the infundibulum was found to be severely damaged by pressure.

Benda [2, 10, 11] has described the pathology of the pituitary gland in mongoloids and has accumulated evidence that hypofunction of the pituitary gland is an important factor in mongolism. The picture of the fatty liver as described above is consistent with this glandular deficiency. However, it is not possible, at the present time, to say definitely whether the pituitary gland, itself, or the infundibulum and stalk may be responsible for the fatty infiltration, as all three have been shown to be involved.

The liver of the infant mongoloid does not show the typical changes observed in the older child and adult. The endocrine balance of the infant is obviously different (for instance, he is usually much underweight) and the difference is reflected in the livers. One-third of the livers from patients under 2 years of age are found to be essentially normal. Two-thirds show congestion which is often extreme. It has been found that dogs deprived of their adrenals show great vascular congestion and hemorrhages around the central veins of the liver.[12] Benda [2] has investigated the pathology of the adrenal glands of mongoloids and has shown that characteristic features of the infant mongoloid (low temperature, fatigue, etc.) may be due to adrenal insufficiency. It seems possible, therefore, that the livers in the young age group reflect this insufficiency. Obviously, the pituitary factor is not dominating at this early age.

SUMMARY

Forty-three livers of mongoloid patients showed a considerable degree of fatty deposit in the majority of cases, often accompanied by a moderate fibrosis. The incidence of fatty livers was about 63 per cent. For patients aged 1 to 28 the incidence of fatty livers was twice as high as in a control series of other mentally deficient patients. It was shown that the fatty deposits are not connected with the occurrence of tuberculosis nor with the degree of obesity in the individual. However, they arise during the second year of life, at the time when the average relative weight of the mongoloid child rises, under the influence of a metabolic disorder. The pathologic picture of fatty infiltration and fibrosis is consistent with hypopituitarism and hypothyroidism which are characteristic for the mongoloid patient.

The mongoloid infant often shows an extremely engorged liver with hemorrhagic necrosis, which may be connected with an adrenal insufficiency.

Fatty infiltration of the same type was found also in several children of a control series of mentally deficient patients. The fatty deposits

were again unrelated to the presence of tuberculosis or obesity. However, in these cases, certain brain lesions, hydrocephalus, microcephalus, and cystic disease, which had produced brain pressure, were found.

I am greatly indebted to Dr. Benda, whose work and helpful advice made this contribution possible.

REFERENCES

1. Brousseau, K. Mongolism. Williams & Wilkins Co., Baltimore, 1928, 210 pp.
2. Benda, C. E. Mongolism and Cretinism. Grune & Stratton, New York, 1946.
3. Benda, C. E. Studies in mongolism. I. Growth and physical development. *Arch. Neurol. & Psychiat.*, 1939, 41, 83–97.
4. Chaikoff, I. L., Entenman, C., Rinehart, J. F., and Reichert, F. L. Development of cirrhosis in the liver of dogs deprived of both pituitary and thyroid glands. *Proc. Soc. Exper. Biol. & Med.*, 1943, 54, 170–171.
5. Graef, I., Negrin, J., and Page, I. H. The development of hepatic cirrhosis in dogs after hypophysectomy. *Am. J. Path.*, 1944, 20, 823–855.
6. Chaikoff, I. L., Eichhorn, K. B., Connor, C. L., and Entenman, C. The production of cirrhosis in the liver of the normal dog by prolonged feeding of a high-fat diet. *Am. J. Path.*, 1943, 19, 9–21.
7. Kraus, E. J. Chronischer Hirndruck und Leberverfettung. *Virchows Arch. f. path. Anat.*, 1937, 300, 617–640.
8. Morgan, L. O. Alterations in the hypothalamus in mental deficiency. *Psychosom. Med.*, 1939, 1, 496–507.
9. Benda, C. E. The central nervous system in mongolism. *Am. J. Ment. Deficiency*, 1940, 45, 42–47.
10. Benda, C. E. Studies in mongolism. III. The pituitary body. *Arch. Neurol. & Psychiat.*, 1939, 42, 1–20.
11. Benda, C. E., and Bixby, E. M. Function of the thyroid and the pituitary in mongolism. *Am. J. Dis. Child.*, 1939, 58, 1240–1255.
12. Banting, F. G., and Gairns, S. Suprarenal insufficiency. *Am. J. Physiol.*, 1926, 77, 100–113.

DESCRIPTION OF PLATES

PLATE 13

FIG. 1. Case 139, 11 years of age. Fatty vacuolation, most severe in the periphery of the lobule. Moderate fibrosis. Hematoxylin and eosin stain. × 95.

FIG. 2. Case 148, 16 years old. Extreme fatty vacuolation, filling the liver uniformly. Heidenhain's azocarmine-aniline blue stain. × 280.

FIG. 3. From the same case as Figure 2. × 890.

FIG. 4. Case 83, 18 years of age. Cell degeneration. Fatty vacuoles in dissolution. Fibrosis is slight and confined to the periportal areas (upper part of field). Hematoxylin and eosin stain. × 740.

Roosen-Runge

Liver in Patients with Mongolism
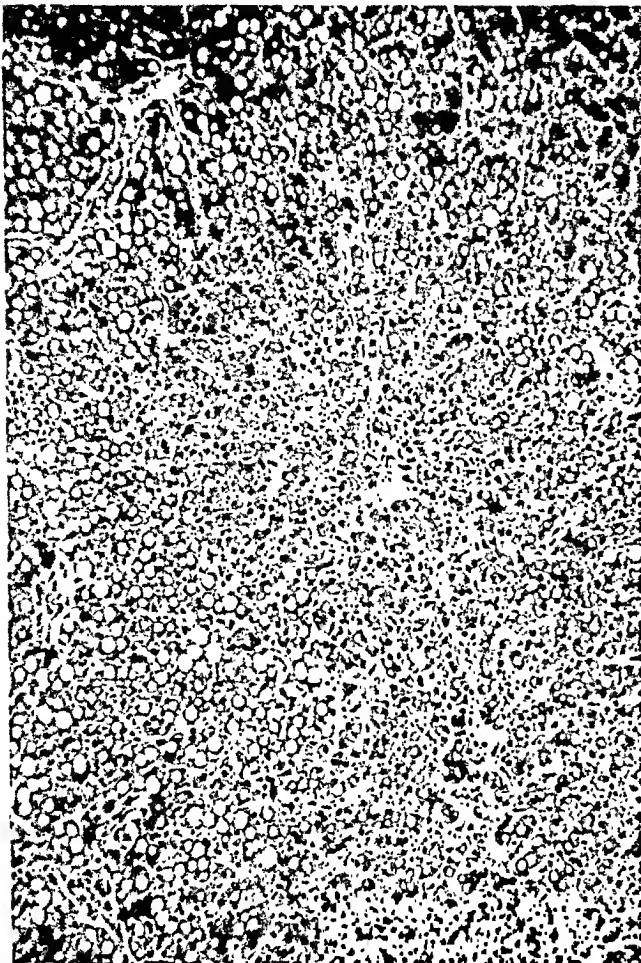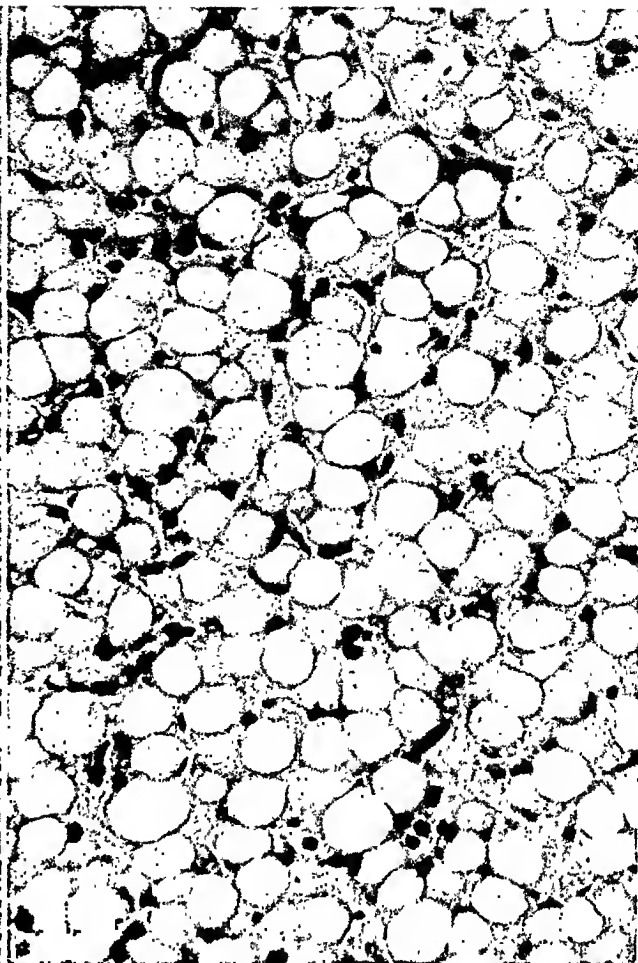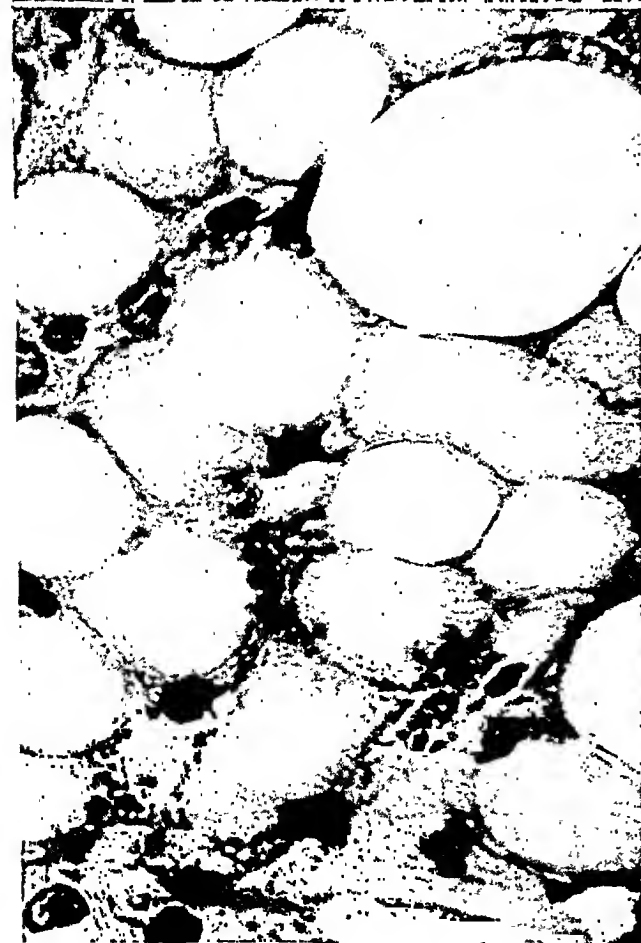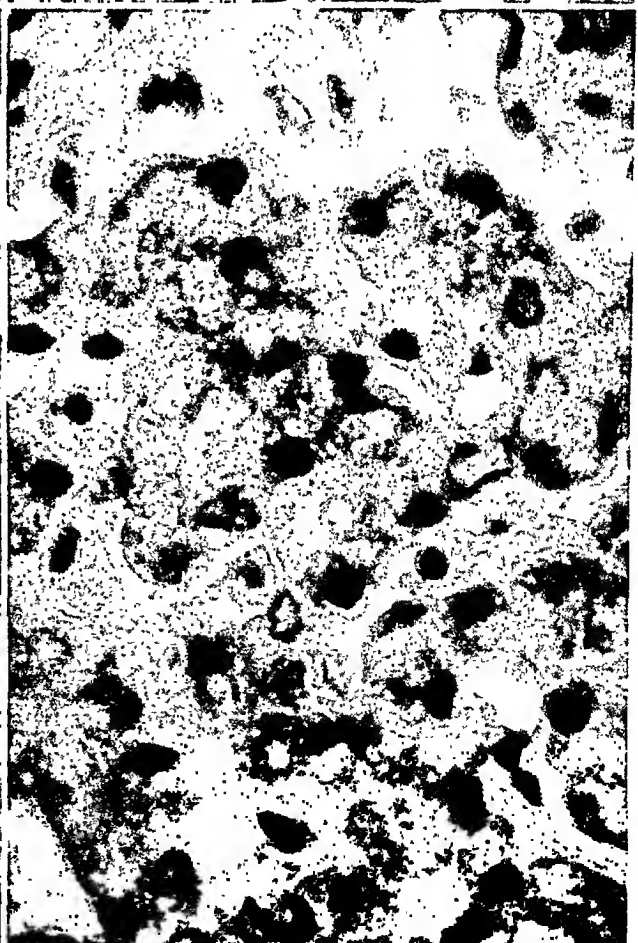
PLATE 14

FIG. 5. Case 58. 16 years old. Pericentral engorgement, periportal vacuolation. Mallory's triple stain. × 130.

FIG. 6. Case C-181. 2½ months of age. Engorgement of all sinusoids. The liver cords are compressed and shrunken. A few of the very rare, fatty vacuoles are seen in the field. Hematoxylin and eosin stain. × 160.

FIG. 7. Case 140. 1 year, 8 months of age. The Kupffer cells are filled with fat (black in the photomicrograph). In the area selected, the liver cells contain little fat. Sudan IV stain. × 240.

FIG. 8. Case 124. 7 years of age. The liver cells contain much fat, except for a one-celled layer which accompanies the vessels and the fibrous tissue around them. The condition of the vessels suggested an old periarteritis nodosa. Sudan IV stain. × 240.
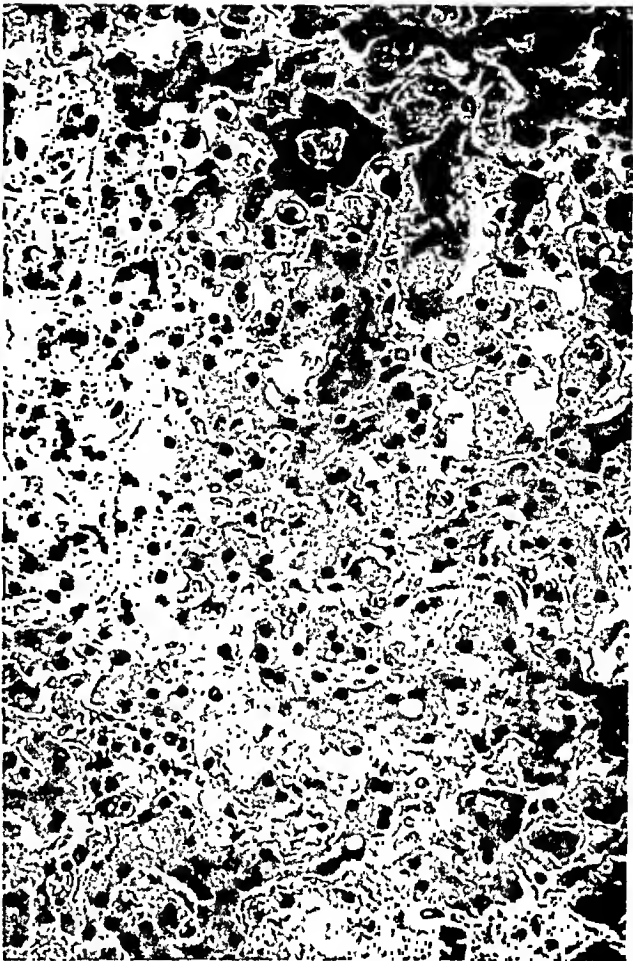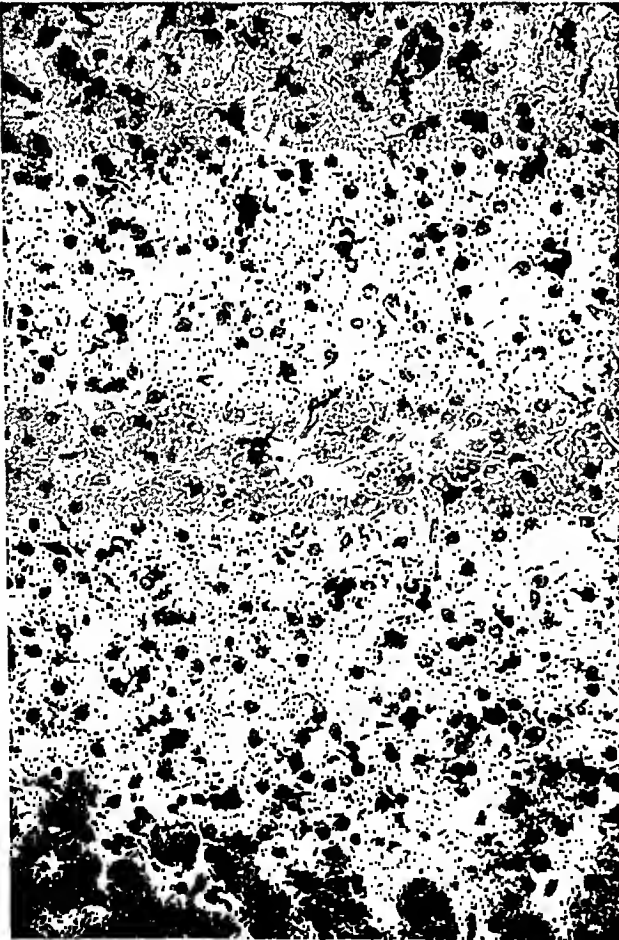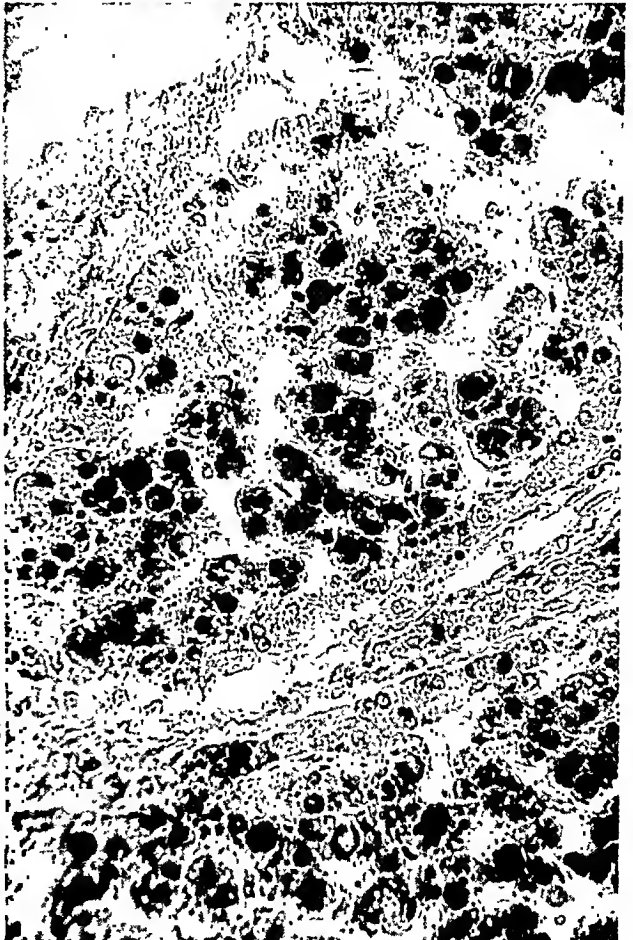
HISTOPATHOLOGY OF MONOCYTIC LEUKEMIA *

PETER A. HERBUT, M.D., and FRANKLIN R. MILLER, M.D.

(From the Clinical Laboratories and Division of Hematology, Department of Medicine, Charlotte Drake Cardeza Foundation, Jefferson Medical College and Hospital, Philadelphia 7, Pa.)

Monocytic leukemia emerged as a distinct entity in 1913 when Reschad and Schilling-Torgau [1] reported the first case. Subsequent medical literature contains an increasing number of case reports and several comprehensive reviews on the subject.[2-8] Most of the publications disclose a detailed account of the clinical manifestations, have excellent descriptions and photomicrographs of monocytic cells as they appear in blood smears, and contain lengthy discussions on the origin of the monocyte. In contrast, however, the histopathologic changes have been reported in detail only sporadically, and in none of the papers in the English language have these descriptions been supported by adequate illustrations. For this reason we are recording the following 8 cases that have been necropsied at the Jefferson Medical College Hospital in the past 7 years.

SUMMARY OF CLINICAL MANIFESTATIONS

Case 1

A white boy, 16 years of age, developed headache 9 days before admission and fever 4 days later following typhoid inoculation. He entered the hospital because of soreness of the teeth and gums. He had oppression in the chest, mass in the left side of the neck, enlarged axillary lymph nodes and spleen, and hypertrophied gums. Studies of the blood showed 2,200,000 erythrocytes per cmm., 64 per cent hemoglobin, and 2,700 leukocytes per cmm. with 64 per cent blast cells. Fever was uncontrolled, leukocytes increased to 210,000 per cmm. and consisted of monocytes and monoblasts, and the bone marrow showed monoblastic hyperplasia. The patient died 26 days after the onset of symptoms.

Case 2

A white man, 30 years old, was well until 8 weeks before admission, during which time he had lost 27 lbs. in weight and developed a dry cough, bleeding gums, and an enlarged spleen. A blood count showed 1,400,000 erythrocytes per cmm., 36 per cent hemoglobin, 43,000 leukocytes per cmm., of which 64 per cent were monoblasts and 5 per cent monocytes, and 44,000 platelets per cmm. Subsequently, the leukocytes increased to 177,000 with 90 per cent monoblasts. Smears of sternal marrow disclosed a hyperplasia of monoblasts, many of which contained Auer's bodies. The patient died 4 months after the onset of symptoms.

Case 3

A 35-year-old white man had a pilonidal sinus removed 4 years previously. This had never healed. He first entered the hospital because of weakness, loss of weight, and pallor. A blood count disclosed 1,200,000 erythrocytes per cmm., 31 per cent

hemoglobin, 1,400 leukocytes per cmm. with 50 per cent lymphocytes, 4 per cent monocytes, and 12 per cent monoblasts. Smears of sternal marrow disclosed oxidase-negative, monocytoid, primitive cells. Five months later he was admitted a third time because of an infection in the upper jaw. The liver was enlarged and there were changes seen roentgenologically in the right femur. The leukocyte count never rose above 2,000 per cmm. Before death there were 41 per cent monocytes and 12 per cent monoblasts in the peripheral blood stream. The duration of illness was approximately 8 months.

Case 4

A white man, 40 years old, had symptoms of peptic ulcer for several years. He was admitted to the hospital in coma with a history of a sore, bleeding mouth and black and blue spots in the skin of 1 week's duration. Examination disclosed bloody spinal fluid, 3,800,000 erythrocytes per cmm. of blood, 79 per cent hemoglobin, 42,000 leukocytes per cmm., of which 20 per cent were monocytes and 68 per cent monoblasts, 22,000 platelets per cmm., and a high proportion of monocytes and monoblasts in the sternal marrow. The patient died 8 days after the onset of acute symptoms.

Case 5

A white man, 43 years old, entered the hospital because of a painful swelling of the left jaw of 1 month's duration. Two weeks previously he had had 9 teeth removed. A blood count showed 3,600,000 erythrocytes per cmm., 69 per cent hemoglobin, and 9,950 leukocytes per cmm. with 53 per cent monoblasts and 5 per cent monocytes. Later the leukocytes increased to 40,000 per cmm. with 77 per cent monoblasts. The gums became hypertrophied, the liver enlarged, and the patient died 2 months after removal of the teeth.

Case 6

A white man, 57 years old, was well until 5 months before admission when he developed a series of furuncles on the face, neck, and abdomen. In the peripheral blood there were (per cmm.) 2,900,000 erythrocytes, 28,000 platelets, and 3,000 leukocytes, of which 5 per cent were monocytes and 4 per cent monoblasts. The sternal marrow showed an increase in monoblasts, some of which contained Auer's bodies. Subsequently, the leukocytes increased to 94,000 with 68 per cent monoblasts and the patient developed slight hypertrophy of the gums. He died 1 year after the first onset of furunculosis.

Case 7

A white man, 62 years old, entered the hospital because of pain in the hips of 6 weeks' duration and a red to purple rash of 2 weeks' duration. A blood count showed 1,800,000 erythrocytes per cmm., 36 per cent hemoglobin, 84,000 platelets per cmm., and 14,200 leukocytes per cmm., with 28 per cent monocytes and 36 per cent monoblasts. The leukocyte count increased to as much as 141,000 per cmm., with 69 per cent monocytes. The sternal marrow showed hyperplasia of primitive cells that were probably monoblasts. The patient died 12 weeks after the onset of pain in the hips.

Case 8

A white man, 70 years old, entered the hospital with anorexia, fatigability and nausea and vomiting, all of which followed an acute upper respiratory infection 6 weeks previously. For 6 years he had had back pain with questionable roentgenologic evidence of lumbar arthritis. Examination disclosed an enlarged spleen, generalized twitching of muscles, and spasm of the muscles of the legs. There were 2,900,000 erythrocytes per cmm. of blood, 54,000 platelets per cmm., and 4,000

leukocytes per cmm., with 7 per cent monocytes and 25 per cent monoblasts. The leukocyte count never rose beyond 6,300 cells per cmm. The patient died in coma 8 weeks after the onset of symptoms.

GROSS PATHOLOGIC OBSERVATIONS

Skin. Cutaneous lesions were present in all cases except 3 and 8. Scattered or innumerable petechiae from pin-point to 2 mm. in diameter, with no predilection for any particular part of the body, were present in all of the positive cases. Usually these were flat and not accompanied by any surrounding induration, but in case 7 the immediately subjacent tissue contained definite firm, gray foci measuring 1 to 2 mm. in diameter. In addition to the petechiae, case 1 disclosed numerous papules measuring 2 to 5 mm. in diameter and raised 1 to 2 mm. above the surrounding surface of skin. Intracutaneous and subcutaneous hemorrhages measuring as much as 14 cm. in diameter were present in cases 1 and 7. Centrally, these were slightly elevated but peripherally they merged gradually with the adjacent normal structures.

Mouth. In cases 2 and 4 the mucous membranes of the mouth, and particularly the gingivae, were covered with bright red and recently coagulated blood. In case 5 there were areas of necrosis without hemorrhage scattered throughout the mouth but unusually prominent over the gums. The gingivae in case 1 were hypertrophied to such a degree that in some areas they completely covered the lateral surfaces of the teeth. Their mucosa was covered with coagulated blood but was not ulcerated and the underlying tissue was moderately firm and pinkish gray. In addition, this case showed numerous petechiae in the mucous membrane of the upper lip and left cheek. Case 3 revealed a superficial erosion of the tip of the tongue and a large ulcer occupying the entire inner portion of the left cheek and the adjacent gums. The floor of the latter contained soft necrotic tissue and the base was reddish gray. There were no oral lesions in cases 6, 7, and 8.

Spleen. Splenomegaly was present in all cases, splenic weights ranging from 270 gm. to 1,290 gm., with an average of 720 gm. The capsules were usually tense, bluish gray, smooth, glistening, and thin. In cases 2, 5, and 8 there was, however, some capsular fibrosis of the central portion of the convex surface, and in cases 2 and 8 these areas were adherent to the diaphragm and anterior abdominal peritoneum. In all cases the edges were rounded. The consistency approached that of liver tissue in cases 1, 2, 4, and 6 (weighing 900, 1,270, 550, and 1,200 gm., respectively), was normal in cases 3 (350 gm.) and 7 (400 gm.), and was considerably softer than normal in cases 5 (270 gm.), and 8 (800 gm.). In all cases the follicular markings were

obscured. The pulp in the firm spleens was deep red and beef-like in appearance, whereas in the soft spleens it was mushy, partially liquefied, and pinkish gray. Depressed, gray to brown infarcts of varying sizes were present in the spleens of cases 1 and 2.

Liver. The liver was slightly or markedly enlarged in all except case 8. Hepatic weights ranged from 1,720 (case 8) to 3,800 gm. (case 6), with an average of 2,480 gm. In all the capsule was smooth, glistening, full or tense, and of normal or decreased thickness. As the organ increased in size the edges became more rounded. All livers were of normal or increased firmness and both on external and cut surfaces they were light or dark reddish brown. Lobular markings were accentuated by thin streaks of gray tissue in half of the cases, while in the remainder they were indistinct. None of the livers showed tumor nodules but in case 2 (weighing 3,000 gm.) there were six abscesses scattered throughout the hepatic parenchyma. They were filled with thick, green, purulent material and measured as much as 1.5 cm. in diameter. The gallbladder and bile ducts were normal.

Bone Marrow. The marrow of the vertebrae was examined in all cases and, in addition, that of the sternum, ribs, and femur in some cases. It was essentially normal in case 8, whereas in the other cases it varied from rather friable, mushy, light reddish brown to firm, dry, pale pink, or gray. In none of the cases was there gross evidence of leukemic penetration of the periosteum, osteolysis, osteosclerosis, or tumor formation.

Lymph Nodes. The lymph nodes were grossly normal in cases 3, 4, and 8. The superficial nodes (cervical, supraclavicular, axillary, and inguinal) were enlarged to as much as 3 cm. in diameter in cases 1, 2, and 6, whereas the deep nodes (mediastinal, hepatic hilar, periaortic, and mesenteric) were enlarged to as much as 5 cm. in diameter in cases 1, 2, 5, 6, and 7. In case 6 the hilar nodes of the liver, measuring 4 cm. in diameter, disclosed some matting, whereas in all other cases the nodes were sharply circumscribed, discrete, well encapsulated, and moderately firm. Cut surfaces were usually homogeneously pinkish gray. In case 7, however, they disclosed minute dark red hemorrhages, and in case 2 several of those at the hilum of the liver contained minute acute abscesses filled with greenish yellow pus. The thymus was enlarged and infiltrated with pinkish gray tissue in cases 1 and 5 and was not identified in the remaining cases.

Gastrointestinal Tract. The gastrointestinal tract was normal in cases 2, 3, and 8. There were petechiae in the serosa of the small intestine in cases 1 and 7, while the mucosa of the stomach and small bowel was congested in case 4. Peyer's patches of the terminal portion

of the ileum were hypertrophied and raised 1 mm. above the surface in cases 1 and 5 and in the latter they were, in addition, superficially ulcerated. The cecum and ascending colon of case 7 disclosed several small submucosal ridges of moderately firm tissue. The perirectal fat tissue in case 6 was indurated and the rectal wall measured 1.5 cm. across. Although the entire wall was infiltrated with gray tissue, the submucosa was proportionately thicker than the remaining layers. The mucosa was thrown into large, firm, pink to red folds, the summits of which were superficially ulcerated. The rectal lumen was reduced to less than 1 cm. in diameter. The large bowel above the constriction was dilated and filled with air and fluid feces. The intestinal tract was otherwise normal.

Kidneys. The kidneys weighed from 100 to 350 gm. with an average of approximately 202 gm. Except for being slightly increased in size, those of cases 3, 4, and 8 were normal. In none was the capsule adherent. In cases 2, 5, and 7 the external and cut surfaces disclosed numerous petechiae throughout the parenchyma. Both cortices and medullas of cases 6 and 7 were considerably paler than normal and the usual demarcations were definitely obscured. In addition gray foci measuring 1 mm. in diameter were found throughout the kidneys of case 6 and the pelves and ureters of cases 1 and 7 were intensely congested and contained recently clotted blood.

Brain. The brain was examined in cases 1, 2, and 4. In case 2 it weighed 1,260 gm. Externally it was normal but within its substance it contained two recent hemorrhages each measuring 0.5 cm. across and situated, respectively, in the white substance of the right frontal and parietal lobes. Each brain in the other 2 cases weighed 1,500 gm. In case 1 it was symmetrically enlarged, whereas in case 4 there was some bulging of the left parietal lobe. Extravasation of blood in the subarachnoid space was slight in case 1 and marked in case 4. In each the gyri were broad and flat and the sulci were obliterated. Coronal sections disclosed the ventricular system in each brain to be filled with fluid and clotted blood. In case 1 there were six separate hemorrhages irregularly distributed throughout the cerebral hemispheres, but there were none in the brain stem and cerebellum. In case 4 most of the left parietal and occipital lobes were destroyed by a massive recent hemorrhage measuring 7.5 by 6 cm. In addition there were scattered small hemorrhages into what remained of the left parietal lobe and into the left temporal lobe. The brain stem and cerebellum were normal.

Other Viscera. There were numerous petechiae in the pericardium of cases 1, 2, and 4, in the endocardium of case 4, and in the pleura of cases 1, 2, and 7. The lungs of all cases showed marked congestion

and edema. Hemorrhages measuring as much as 6 cm. in diameter
were irregularly distributed throughout the lungs in cases 1, 3, 4, and
6, and raised, gray, indefinitely circumscribed foci measuring up to 1
cm. across were present in those of cases 1, 3, 6, and 8. The remaining
thoracic and abdominal organs showed no contributory changes.

MICROSCOPIC PATHOLOGIC OBSERVATIONS

Sections of all organs were stained with hematoxylin and eosin. In
addition, triplicate sections of the spleen, lymph nodes, and bone mar-
row of all cases and of the liver of some cases were stained with hema-
toxylin and eosin, Giemsa's stain, and Foot's reticulum stain. Stains
for oxidase granules in histologic sections were carried out on tissue
from some of the cases using, as control material, tissue from a case of
myeloid leukemia stored for approximately the same time. None
showed peroxidase granules, probably due to the fact that all material
was too old.

The Monocyte in Histologic Sections

The monocytic cells within vessels were more uniform than were
those infiltrating the tissues. They were round or oval, and one and
one-half to three times the diameter of erythrocytes (Figs. 1 and 2).
The cell borders were usually distinct and sharp but occasionally they
were ill defined and fuzzy. The cytoplasm occupied one-eighth to three-
quarters of the cell volume and with hematoxylin and eosin stained
deep pink, whereas it was stained light blue by the Giemsa method.
Sometimes the cytoplasm was proportionately more abundant and con-
tained phagocytosed nuclear material, brown pigment, and erythrocytes
(Fig. 3). The nuclei were ordinarily relatively large and were round,
oval, or irregular. The irregular nuclei often had a single indentation
giving a typical horseshoe-shaped appearance, but also they fre-
quently contained two or more deep indentations which gave the ap-
pearance of definite lobulations. Occasionally the nuclear material was
irregularly twisted upon itself, necessitating fine focusing to distinguish
the convolutions. The nuclear margins were always distinct but the
nucleoplasm generally stained very lightly and contained chromatin
grouped in small, irregular aggregations, sometimes connected by fine,
almost imperceptible threads. Nucleoli were not seen in any of the
intravascular monocytic cells.

The monocytic cells within the alveolar spaces of the lungs possessed
essentially the same characteristics as those within vessels except that
they were slightly larger, somewhat more irregular, showed more
phagocytosis, and many mitotic figures (Fig. 4). The nuclei were per-

haps more darkly stained and generally more irregular than were those in the circulating blood. Nucleoli were not seen.

In tissues the cells showed considerably more pleomorphism. When they were more mature and less tightly packed they closely resembled those in the pulmonary alveolar exudate and circulating blood. When, however, they were less mature and more crowded, all gradations were seen from the round or oval cells just described to large spindle-shaped, polyhedral or irregular forms which were indistinguishable from regular reticulum cells (Fig. 5). Often long, thin, fibrillary processes projected from the angles of the more irregular cells and were directly continuous with the supporting reticulum (Figs. 6 and 7). At other times the sinus endothelial cells, particularly in the bone marrow, were swollen and showed varied degrees of detachment both intravascularly and extravascularly until the cytoplasm was completely separated (Fig. 8). The nuclei of all cells mentioned were similar in staining qualities but varied in size and external configuration. In the reticulum cells and in the more immature spindle and irregular monocytic cells they were considerably larger than in the round and oval circulating monocytes. Often they were of irregular shape, assuming triangular and rectangular configurations. They showed few twists and indentations, and rarely assumed the characteristic horseshoe shape. Occasionally there appeared to be two or three separate nuclei piled up in a single cell to produce what closely resembled Sternberg-Reed giant cells. One or two nucleoli were seen in some of the more immature cells when stained by the Giemsa method. They could not be definitely identified in the routine sections stained with hematoxylin and eosin. Mitotic figures were numerous among the immature cells but were less frequent or entirely absent among the more mature monocytes.

Skin. Histologic sections of the skin were available from cases 1, 2, 4, 6, and 7. The epidermis was normal. The immediately subjacent dermis was edematous but the remainder was dense and collagenous. Hemorrhagic extravasation was present in all cases, reaching a maximum degree in case 4. Usually, small collections of erythrocytes were insinuated between the collagen fibers throughout the dermis. Sometimes they were adjacent to capillaries but at other times they appeared to be independent of vessels. In case 4 there were, in addition, massive hemorrhages into the subcutaneous tissue. Infiltrations of leukocytes were also present in all cases. In the superficial portions of the dermis, particularly in the edematous zone, they usually surrounded small capillaries, but in the deeper layers this relationship was less apparent. The most severe infiltrates were grouped around hair shafts and sebaceous and sweat glands (Fig. 9). Whereas in some instances

(case 1) the infiltrating leukocytes were all of the monocytic variety, in others (case 2) there were present also a few myeloid cells. In all cases erythrocytic extravasation appeared to be entirely independent of the leukocytic foci.

Gingivae. Sections of the gums were available from case 1. The mucosa was intact and, except for some vacuolization of the prickle cell layer, was normal. The immediately subjacent submucosa was edematous but contained only a few empty capillaries and was sparsely infiltrated with round or oval monocytes (Fig. 10). In the deeper tissues, however, the monocytes were so densely packed that they obliterated all the normal structures with the exception of a few strands of striated muscle. In these areas the monocytic cells were polygonal and quite irregular, showed scattered mitotic figures, and were not particularly disposed about blood vessels. Throughout the sections there were only a few widely separated and insignificant foci of extravasated erythrocytes.

Spleen. Histologically, the spleens of cases 1, 3, 5, 6, and 7 were essentially the same. In each the capsule was thin and the trabeculae were decreased relatively in number and size. These spleens contained varied numbers of monocytes and erythrocytes. Normal follicles were practically nonexistent (Fig. 11). In some the arterioles were quite prominent and were surrounded not by lymphocytes but by cuffs of large, irregular, immature monocytes that were often in a state of mitosis (Fig. 12). Sprinkled among these cells were a few polymorphonuclear leukocytes and lymphocytes. The periphery of each periarteriolar collection was sharply separated from a pulp infiltrated with somewhat more regular and smaller monocytic cells. The infiltration of the latter was so dense that the underlying sinusoidal spaces could not be recognized. There was, however, no apparent hyperplasia of the reticulum of the pulp and erythrocytes were not plentiful. Eosinophils were present in the spleens of cases 5 and 7. The former, in addition, disclosed several areas containing relatively large single or multinucleated giant cells that were indistinguishable from Sternberg-Reed cells (Fig. 13). Sections from the spleen of case 2 differed from the foregoing in that they were less homogeneous. The normal periarteriolar collections of lymphocytes were replaced with solid cuffs of hyperplastic reticulum, between the meshes of which were many monocytes, fewer phagocytes laden with golden brown pigment, polymorphonuclear leukocytes, and lymphocytes (Figs. 14 and 15). The pulp was less solid than in the previously described cases and in many areas there was a definite increase of fine reticulum with which the monocytes were intimately bound and often connected by fine processes. The

spleen of case 4 was even less homogeneous. Normal follicles were present in some areas while in other areas both the follicles and the pulp were completely replaced with a solid infiltration of monocytes with fewer myeloid and erythroid cells. Between these solid areas the reticulum showed much hyperplasia and widely patent sinusoids (Fig. 16). The endothelium of the latter showed patchy or continuous hyperplasia and an extrusion of some of the lining cells into the lumen to form free monocytes. When less densely packed, the monocytic cells in the intersinusoidal spaces were seen to be intimately connected with the underlying reticulum (Fig. 17). In some areas the presence of mononuclear giant cells and eosinophils presented a picture not unlike that of Hodgkin's disease. The spleen of case 8 was so crowded with erythrocytes that the underlying architecture was completely obliterated. Beneath the capsule, however, there were collections of large irregular monocytes and fewer lymphocytes, plasma cells, and polymorphonuclear leukocytes which, but for the absence of eosinophils, resembled Hodgkin's disease.

Liver. The distribution of the leukemic infiltrates in the liver was not uniform. In cases 1, 2, 5, and 6 the portal areas were decidedly more involved than were the lobules, but both participated in the process (Fig. 18). In the former there were varied degrees of reticulum hyperplasia. This reached a maximum degree in case 1 where the cells around the vessels often assumed a more or less whorled formation. Throughout these areas mitotic figures were quite common and there were all transitions from large, polyhedral cells with abundant cytoplasm and long, fibrillary processes to the relatively mature, round or oval monocytes. Other leukocytic cells were almost entirely absent. Perisinusoidal edema throughout the lobules rendered the sinusoids particularly conspicuous. Some lining endothelial cells showed various degrees of swelling until they were completely detached to form intrasinusoidal phagocytes (Figs. 19 and 20). Both Kupffer's cells and the intrasinusoidal phagocytes contained ingested brown pigment and nuclear fragments. Other swollen endothelial cells showed no phagocytosed material and appeared to be gradually extruded into the lumina as monocytes in varying numbers and in various stages of maturity. Mitotic figures were present only occasionally. The nuclei of the swollen endothelial cells were identical with those of both the intrasinusoidal phagocytes and the monocytes. The liver cells were somewhat atrophic but otherwise were well preserved except in case 2 where they showed severe central degeneration. Lipoidosis was patchy, and mitotic figures were present in some of the hepatic cells of case 1. Infiltrations of leukemic cells in the livers of cases 4 and 7 were practi-

cally confined to the liver cords with almost no involvement of the portal canals (Fig. 21). In case 7, however, only about 20 per cent of the cells were monocytic while the rest were of the myeloid series. The livers of cases 3 and 8 were similar in that the distribution of leukemic cells in the portal areas and the sinusoids was about equal. In case 3 it was less extensive than in case 8. In the former, however, most of the cells were monocytes, whereas in the latter only about 10 per cent were monocytic and the rest were myelocytic. Convincing transitions from Kupffer's cells to free monocytes were demonstrable in the liver of case 3 but not in the livers of cases 4, 7, and 8.

Bone Marrow. The bone marrow of the sternum was consistently more involved than was that of the ribs, vertebrae, and femora. It was extremely dense in cases 1 and 7 (Figs. 22 and 23), and least of all in case 8. The monocytic cells were most immature in cases 1 and 7 where they constituted from 85 to 100 per cent of the marrow cells. In case 1 all gradations could be traced from ordinary reticulum cells and sinus endothelial cells to mature round or oval monocytes. Elongated and irregular polygonal cells with long processes attached both to sinus endothelium and underlying reticulum were particularly conspicuous (Figs. 5 and 6). In case 7, although the cells were irregular, fibrillary processes were absent. In both cases fat cells were entirely crowded out, but between the leukemic cells there were scattered remnants of myeloid, erythroid, and megakaryocytoid cells. In cases 2, 3, 4, 5, and 6 the marrow, although diffusely involved, was less densely packed than it was in the aforementioned cases, and there were occasional scattered fat cells. The number of monocytic cells, as compared with other marrow elements, varied from 60 to 100 per cent. In all of these cases, however, the leukemic cells were more mature and were thus usually round or oval as compared with the more prevalent immature and irregular cells seen in the 2 former cases; hence fibrillary processes and transitions to reticulum cells were not apparent.

The marrow of case 8 was in a separate category. The right femur, vertebrae, ribs, and sternum were examined histologically and were all essentially similar. The general architecture of the marrow was little disturbed, showing an abundance of fat and other marrow cells. Throughout the sections, however, there were conspicuous reticulum cells and huge monocytes (Fig. 24). Usually the leukemic cells were sparsely scattered and represented not more than 10 per cent of all cells, but sometimes they were collected into small foci in which they constituted about 80 per cent of the marrow cells. Many of the monocytes were irregularly polygonal with pointed angles from which fibrillary processes emerged to join the underlying reticulum. Their cyto-

plasm was relatively scanty and even in sections stained with hematoxylin and eosin was light blue. The nuclei were lightly stained, round or oval, and similar to those of other monocytic cells except that they were considerably larger. Mitotic figures were abundant. Although definite connections of these cells with the sinus endothelium could not be demonstrated, the nuclei of swollen endothelial cells were very similar to those of the monocytic cells. In none of the cases was the cortical bone penetrated or the periosteum stimulated to produce new bone.

Lymph Nodes and Thymus. Although the lymph nodes of all cases were involved in the leukemic process, the degree of infiltration varied considerably. Those of case 3 were least affected. Follicular architecture was fairly well preserved but scattered throughout the nodes were round or oval monocytic cells comprising about 25 per cent of all free cells. There were present, also, numerous phagocytes containing golden brown pigment and only very few eosinophils. The nodes of case 4 -were slightly more involved. The follicles were still recognizable and consisted of 100 per cent lymphocytic cells. Between these, however, there were broad, branching bands of densely packed reticulum cells which showed transformation to both monocytes and large mononuclear phagocytes (Fig. 25). The nodes of cases 2, 5, 6, and 7 were essentially similar. Except for a few scattered atrophic follicles, the normal architecture was completely replaced by a diffuse infiltration with monocytic cells (Fig. 26). They were round or oval and showed no processes or attachments either to the reticulum or sinus endothelium. The reticulum was increased only focally around the thicker vessels in the medulla of some of the nodes (Fig. 27). The capsules were uniformly infiltrated with monocytic cells. In cases 2 and 7, however, the presence in the less densely infiltrated areas of a few plasma cells, polymorphonuclear leukocytes, eosinophils, and binucleate or multinucleate giant cells produced an appearance very similar to that of Hodgkin's disease (Fig. 28). The lymph nodes of case 1 were perhaps most severely involved. The cortices were completely replaced with closely packed monocytic cells which were nevertheless round or oval. Mitotic figures were abundant. The medullas, particularly around the thicker blood vessels, showed a severe degree of reticulum hyperplasia with numerous polygonal and elongated cells that often assumed a sarcomatous appearance. From these there were transitions to typical monocytes, but in many instances it was impossible to label the cells as one or the other with any degree of assurance. Although generally the medullas were sharply separated from the cortices by a fine connective tissue capsule, in several areas the latter was interrupted and the cells in the two zones were freely intermingled (Fig.

29). The nodes of case 8 were replaced with less densely packed cells than were those of the other cases. Huge single and multinucleated giant cells indistinguishable from Sternberg-Reed cells were very conspicuous everywhere. Smaller monocytes, although present, were less prevalent (Fig. 30). The reticulum was generally increased and often was intimately connected with the irregular monocytic cells. The presence of phagocytes, few plasma cells, myeloid cells, and lymphocytes produced a picture somewhat resembling that of a "sarcomatous" type of Hodgkin's disease. Sections of the thymus from cases 1 and 5 were examined and in each they were similar to the sections of lymph nodes from the respective cases (Fig. 31).

Intestines. Involvement of the lymphoid apparatus of the intestine paralleled that of the lymph nodes. In some of the cases there was little or no infiltration with leukemic cells. In others, as in case 5, the follicles were normal but the pulp disclosed a definite increase in monocytes. Except for ulceration of the overlying mucosa, there were no other changes in the bowel wall. Peyer's patches in case 1 showed the most severe involvement. The lymph follicles were prominent but were not normal. The germinal centers of all of them were enlarged and completely replaced with large, irregular reticulum cells and monocytes (Fig. 32). At the periphery they were surrounded by a thin, compressed rim of lymphocytes, beyond which the pulp was again entirely replaced with reticulum cells and monocytes. The covering mucosa, as in case 5, was ulcerated. In case 7 the mucosa of the small intestine was intact but congested. The submucosa was thickened and edematous in patchy areas and was sparsely infiltrated with monocytes. Edema and fibrosis of the submucosa reached its maximum degree in the rectum of case 6 where it measured 1 cm. thick. Both the submucosa and serosa were heavily infiltrated with monocytes but the muscle contained only scattered cells.

Kidneys. The kidneys of cases 2, 4, 5, 6, and 7 were involved in the leukemic process. The infiltrates of monocytic cells were primarily interstitial and in the cortex, and only when very severe was the medulla involved. In cases 2 and 4 the foci were small. They were larger in cases 5 and 6 (Fig. 33) and in case 7 there was a diffuse infiltration of both the cortex and medulla. When the foci were small, the renal architecture was not disturbed, but as they increased in size there was first atrophy and later complete replacement of all the normal structures.

Other Organs. There were submucosal hemorrhages and surrounding monocytic infiltrations in the ureters of case 7 and the bladder of case 1, and a diffuse interstitial infiltration with leukemic cells in the

testes of cases 1 and 7. In the heart there were subendocardial foci of monocytes in cases 4, 6, and 7, pericardial foci in cases 1 and 7, and myocardial foci in case 7. Congestion and edema were present in the lungs of all cases and hemorrhages in those of cases 1, 4, 5, and 6. Exudate of monocytic cells, which appeared somewhat larger than those in the circulating blood, was present in the septa and alveolar spaces in cases 1, 2, 3, 5, 6, and 7, in reality producing a monocytic pneumonia (Fig. 4). In addition to the monocytes, some of the exudate contained scattered fibrin threads and a few large phagocytic cells exhibiting ingested débris. In the adrenals, leukemic infiltrations of monocytic cells were present in the inner zones of the cortices, in the medullas, or in the periadrenal connective tissue of cases 1, 2, 6, 7, and 8. There was a diffuse interstitial infiltration of monocytes in the pancreas of case 7. The remaining thoracic and abdominal organs showed no contributory changes. Sections of the brain in each of the cases in which it was examined (1, 2, and 4) disclosed monocytic cells filling the vessels and extravasated beneath the meninges and into the areas of hemorrhage. Occasionally, there were scattered foci within the white matter, apparently independent of blood vessels. About some of the arterioles in case 2 there was an adventitial proliferation of reticulum cells and what appeared to be transformation of these cells into monocytes (Fig. 34). The brain substance around the hemorrhages was edematous and vacuolated but showed no attempt at repair.

COMMENT

Participation of the reticulo-endothelial system in monocytic leukemia has been variously reported. Some authors have not commented upon it, others have noted an increase in reticulum cells,[2,3,7-18] and still others have described an absence of their proliferation.[11,14,15] In some of our cases there was a definite hyperplasia of the reticulo-endothelial system with a traceable transformation of the cells into monocytes, while in other cases such changes were not demonstrable. The explanation for this disparity is apparently two-fold. First, the age of the patient appears to be important. It seems as though the younger the individual (cases 1 and 2) the more severe and profuse will be the response and, conversely, the older the individual (case 8) the less extensive will be the proliferative process. Secondly, of equal importance and coupled with the age of the patient appears to be the amount and duration of the stimulus. When the disease is fulminating, and presumably when the stimulus is more potent, there will be such a rapid proliferation of reticulum cells that their maturation into mono-

cytes cannot keep pace, resulting in an accumulation in those locations where they are usually most abundant. When, however, the disease is more protracted, and presumably when the stimulus is less, there will be a slower degree of proliferation of reticulum cells resulting in their more subtle and complete transformation into monocytes. Under these circumstances there is neither an evident accumulation of reticulum cells nor is there a traceable differentiation of these cells into monocytes.

Although Kupffer's cells are a part of the reticulo-endothelial system, a review of the literature discloses that their transformation into monocytes has never been satisfactorily demonstrated. We believe that in some of our cases such a transition was unquestionably encountered. A careful study of many sections of the livers disclosed a swelling of the nuclei and cytoplasm of the Kupffer cells with a gradual bulging into the sinusoidal lumina and eventually a complete detachment to form free intrasinusoidal cells. While some of these became ordinary phagocytes, others became rounded off to form circulating monocytic cells. As would be expected, the nuclei of the swollen endothelial cells, phagocytes, and monocytes were identical. Mitotic figures, however, were only rarely seen in the Kupffer cells.

Several authors have noted a striking histopathologic similarity between subacute and chronic monocytic leukemia and Hodgkin's disease.[4,8,16,17] While we, too, encountered such a resemblance in the spleens of cases 4, 5, and 8, and in the lymph nodes of cases 2, 7, and 8, it is to be noted that in all of these the disease was fulminating, and that in case 6, in which the disease was more protracted, there was no such similarity. It is possible, therefore, that the various types of leukocytic cells producing a picture of Hodgkin's disease are found when the stimulator or stimulators first hit the tissues and when all cells proliferate. Later, when the action of the specific stimulator becomes stabilized, only one type of cell is produced—the monocyte—and this floods and replaces the previous heterogeneous mixture of leukocytic cells with a homogeneous one.

Finally, it is noteworthy that hemorrhages and leukemic infiltrations were present in each brain examined. In one of these (case 2) the hemorrhage was small and inconsequential, but in the remaining two (cases 1 and 4) it undoubtedly contributed to, or was the immediate cause of, death. In a recent review of the brain in leukemias, Leidler and Russell [18] studied a total of 67 cases, of which only 3 were of the monocytic variety. In 62 there were leukemic infiltrations and/or hemorrhages, and the latter were of sufficient degree to be the immediate or contributory cause of death in 27 cases or 40 per cent.

SUMMARY AND CONCLUSIONS

By a detailed study of the histopathologic changes in 8 cases of monocytic leukemia, it was shown that reticulo-endothelial cells are unquestionably the precursors of monocytes. Their demonstrable proliferation and transitions to the freely circulating cell appear to depend upon the age of the patient and the degree of stimulation. A fulminating course in a young person will produce extensive reticulum hyperplasia because the maturation of the cells cannot keep pace with proliferation. In an older person a similarly acute course will produce less hyperplasia because the tissues are less adaptable to proliferation. In the latter, as well as in protracted cases, there is a more subtle and complete transformation of the reticulo-endothelial cells into monocytes and the transitions are more difficult to demonstrate.

Like all reticulo-endothelial cells, the Kupffer cells of the liver were seen to participate in the formation of monocytes in some of our cases. All transitions from initial swelling to complete extrusion into the sinusoidal lumina were encountered.

In our cases a heterogeneous accumulation of leukocytic elements, producing a picture resembling Hodgkin's disease, was found in the acute cases. This is in contrast to the reports in the literature in which this feature is described as occurring in the subacute and chronic forms of the disease.

The brain was examined in 3 cases. In each of these there were leukemic infiltrates and hemorrhages. The latter were of sufficient degree to be a contributory or immediate cause of death in 2 cases.

REFERENCES

1. Reschad, H., and Schilling-Torgau, V. Ueber eine neue Leukämie durch echte Uebergangsformen (Splenozytenleukämie) und ihre Bedeutung für die Selbständigkeit dieser Zellen. *München. med. Wchnschr.*, 1913, 60, 1981–1984.
2. Dameshek, W. Acute monocytic (histiocytic) leukemia. Review of the literature and case reports. *Arch. Int. Med.*, 1930, 46, 718–740.
3. Clough, P. W. Monocytic leukemia. *Bull. Johns Hopkins Hosp.*, 1932, 51, 148–177.
4. Doan, C. A., and Wiseman, B. K. The monocyte, monocytosis, and monocytic leukosis. A clinical and pathological study. *Ann. Int. Med.*, 1934–35, 8, 383–416.
5. Mercer, S. T. The dermatosis of monocytic leukemia. *Arch. Dermat. & Syph.*, 1935, 31, 615–635.
6. Osgood, E. E. Monocytic leukemia. Report of six cases and review of one hundred and twenty-seven cases. *Arch. Int. Med.*, 1937, 59, 931–951.
7. Freeman, H. E., and Koletsky, S. Cutaneous lesions in monocytic leukemia. Report of two cases with pathologic study. *Arch. Dermat. & Syph.*, 1939, 40, 218–240.
8. Evans, T. S. Monocytic leukemia (general review of the subject). *Medicine*, 1942, 21, 421–456.

9. Foord, A. G., Parsons, L., and Butt, E. M. Leukemic reticulo-endotheliosis (monocytic leukemia), with report of cases. *J. A. M. A.,* 1933, 101, 1859–1866.

10. Jetter, W. W. Acute monocytic leucemia. *Med. Rec.,* 1937, 146, 429–434.

11. Watkins, C. H., and Hall, B. E. Monocytic leukemia of the Naegeli and Schilling types. *Am. J. Clin. Path.,* 1940, 10, 387–396.

12. Giffin, H. Z., and Watkins, C. H. The distinction between splenic anemia and subleukemic splenic reticulo-endotheliosis. *Am. J. M. Sc.,* 1934, 188, 761–767.

13. Sydenstricker, V. P., and Phinizy, T. B. Acute monocytic leukemia. A case with partial autopsy. *Am. J. M. Sc.,* 1932, 184, 770–777.

14. Campbell, A. C. P., Henderson, J. L., and Croom, J. H. Monocytic leukaemia with myeloid hyperplasia and localised tumour formation. *J. Path. & Bact.,* 1936, 42, 617–634.

15. Sweany, H. C., and Cannemeyer, W. Monocytic leukemia. *Arch. Path.,* 1941, 32, 429–440.

16. Damashek, W. Proliferative diseases of the reticulo-endothelial system. II. Aleukemic reticulosis. Report of a case. *Folia haemat.,* 1933, 49, 64–85.

17. Cabot case no. 23021. *New England J. Med.,* 1937, 216, 65–70.

18. Leidler, F., and Russell, W. O. The brain in leukemia. A clinico-pathologic study of twenty cases with a review of the literature. *Arch. Path.,* 1945, 40, 14–33.

DESCRIPTION OF PLATES

PLATE 15

FIG. 1. Circulating monocytes in a pulmonary blood vessel of case 7, with many spherical cells with round or indented nuclei and a moderate amount of cytoplasm. Hematoxylin and eosin stain. × 400.

FIG. 2. Circulating monocytes of case 7 at a higher magnification, showing indented, round, or irregular nuclei with lightly stained chromatin. Hematoxylin and eosin stain. × 900.

FIG. 3. From a vein in the liver of case 1, showing closely packed monocytes and large intravascular macrophages containing ingested material. Hematoxylin and eosin stain. × 400.

FIG. 4. Monocytic exudate into the lung of case 1. The cells are slightly larger than those within vessels. They are round, have distinct borders and nuclei, and some are in mitosis. A few phagocytic cells are present also. Hematoxylin and eosin stain. × 400.

FIG. 5. From the thymus from case 1, showing an intimate mixture of reticulum cells and monocytes in various stages of maturity. Hematoxylin and eosin stain. × 400.

FIG. 6. Another area from the thymus from case 1, showing monocytes with long tail-like processes attached to the related reticulum. Hematoxylin and eosin stain. × 900.
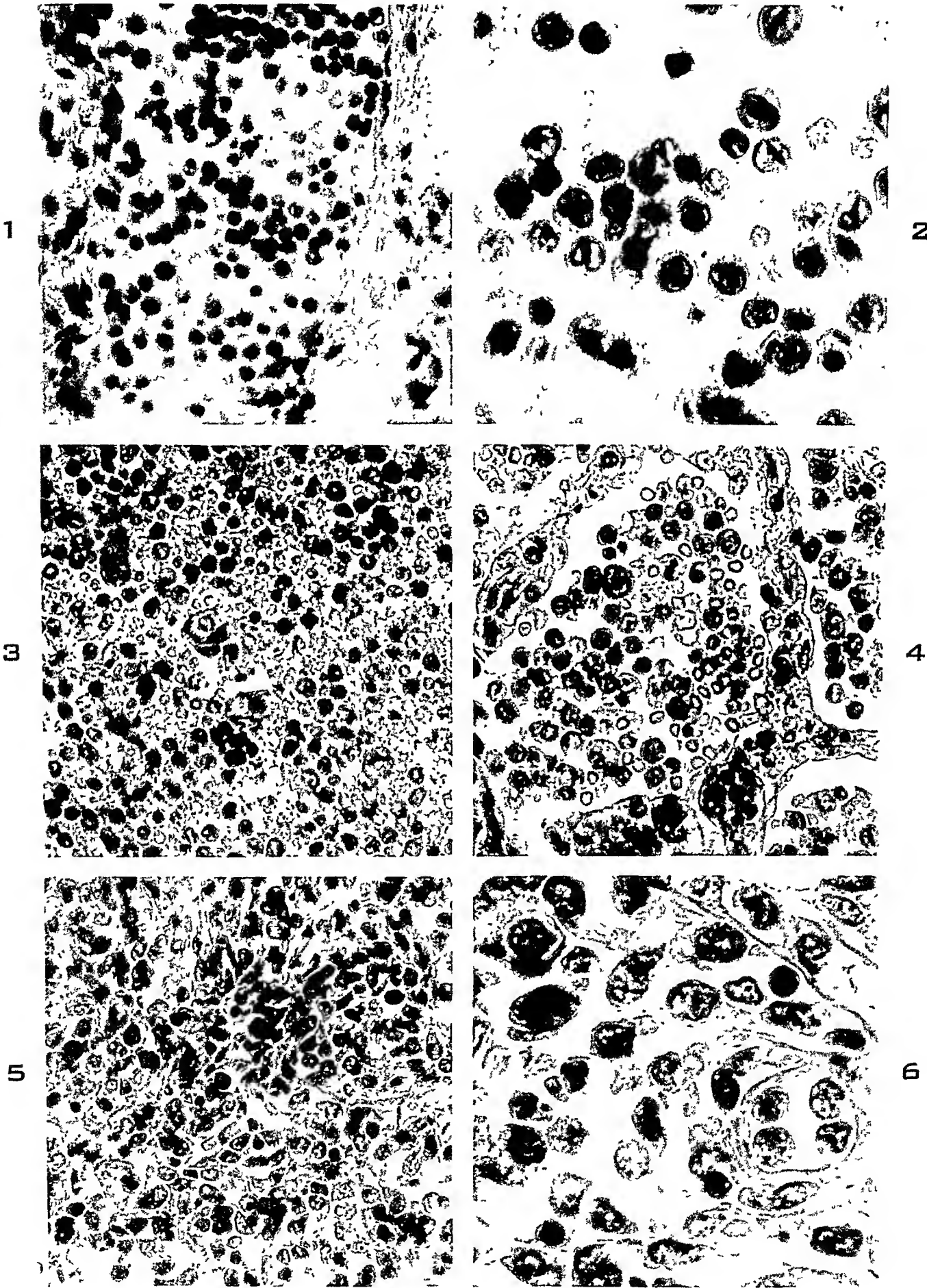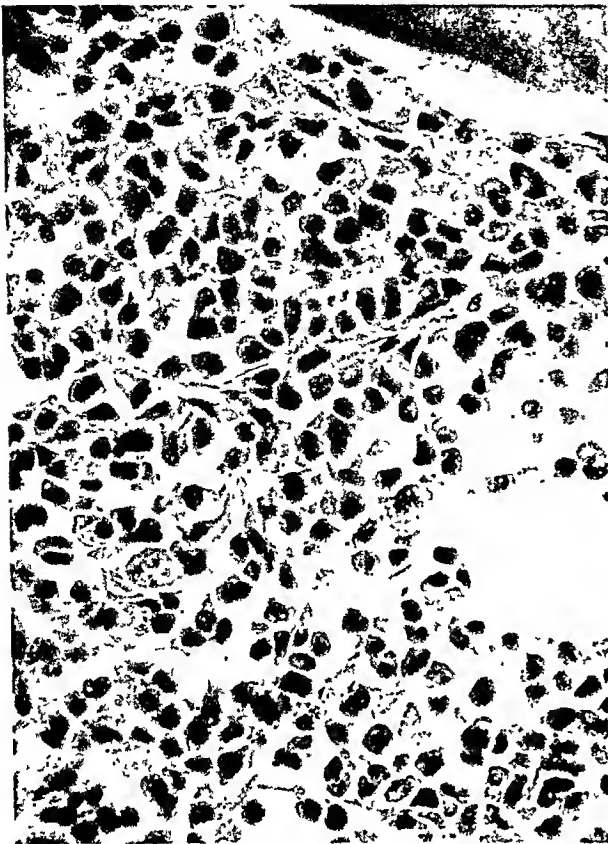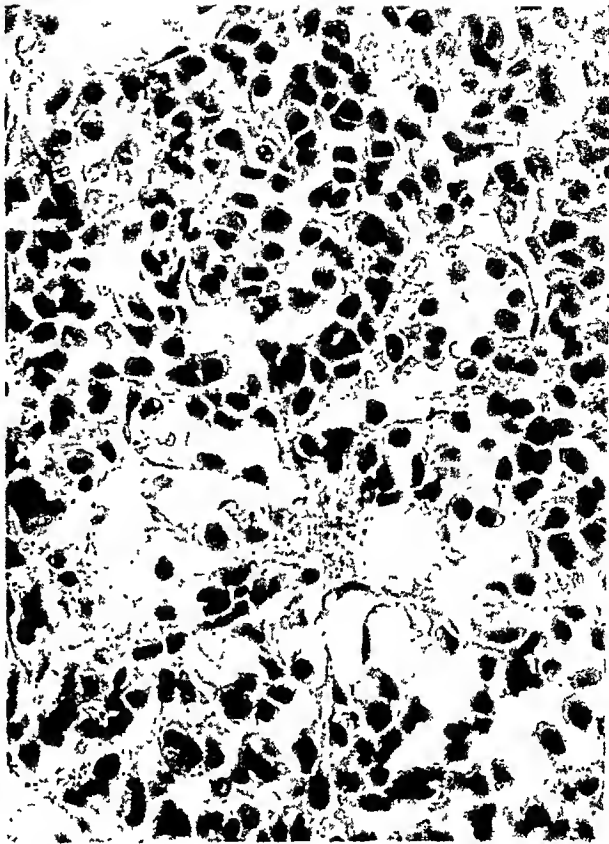
PLATE 16

FIG. 7. The vertebral bone marrow from case 1, showing some monocytes intimately connected with the underlying reticulum. Hematoxylin and eosin stain. × 400.

FIG. 8. Another area from the vertebral bone marrow of case 1 shows bulging of the sinus endothelium and extrusion to form monocytes. Hematoxylin and eosin stain. × 400.

FIG. 9. Section of the skin from case 7, showing monocytic infiltration about a hair shaft. Hematoxylin and eosin stain. × 50.

FIG. 10. Section of the gingiva from case 1, showing intact epithelium and edematous submucosa infiltrated with monocytes. Hematoxylin and eosin stain. × 50.
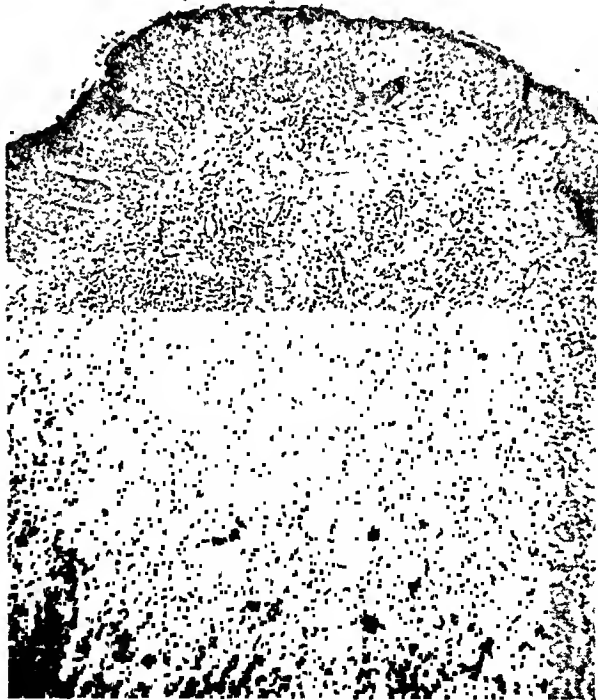
PLATE 17

FIG. 11. The spleen from case 6 shows complete effacement of the normal architecture. Hematoxylin and eosin stain. × 50.

FIG. 12. Section of the spleen from case 1, showing an arteriole surrounded by immature monocytes sharply demarcated from the pulp. Some of the cells are in mitosis. Scattered sparsely among the monocytic cells there are a few polymorphonuclear leukocytes and lymphocytes. Hematoxylin and eosin stain. × 200.

FIG. 13. The spleen from case 5 presents a picture resembling Hodgkin's disease. Mononuclear and multinuclear giant cells of the Sternberg-Reed type, eosinophils, and other leukocytic cells are present. Giemsa's stain. × 200.

FIG. 14. Section of the spleen from case 2, showing marked periarteriolar reticulum cell hyperplasia. Hematoxylin and eosin stain. × 50.
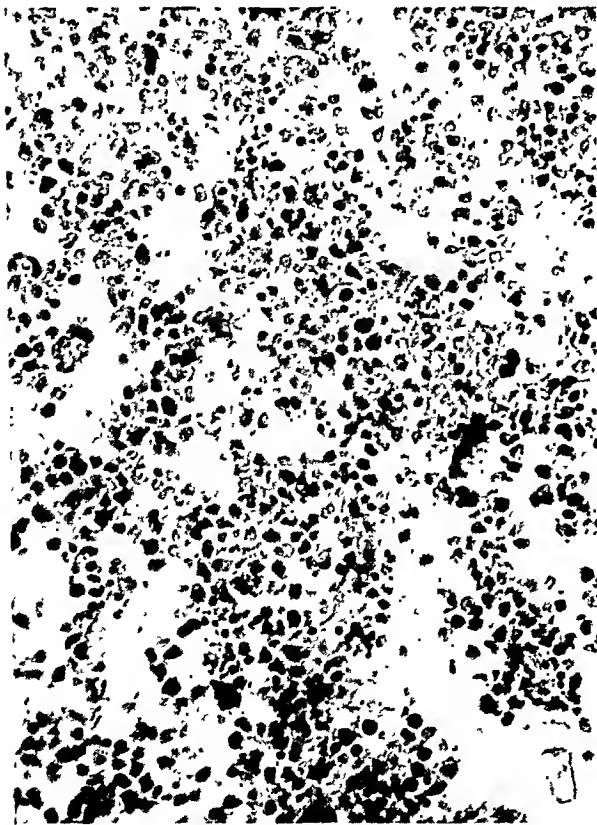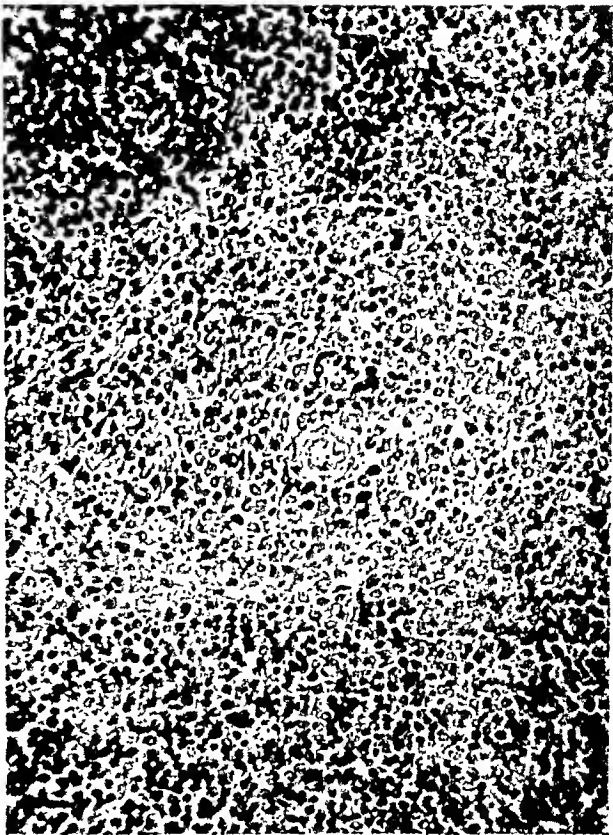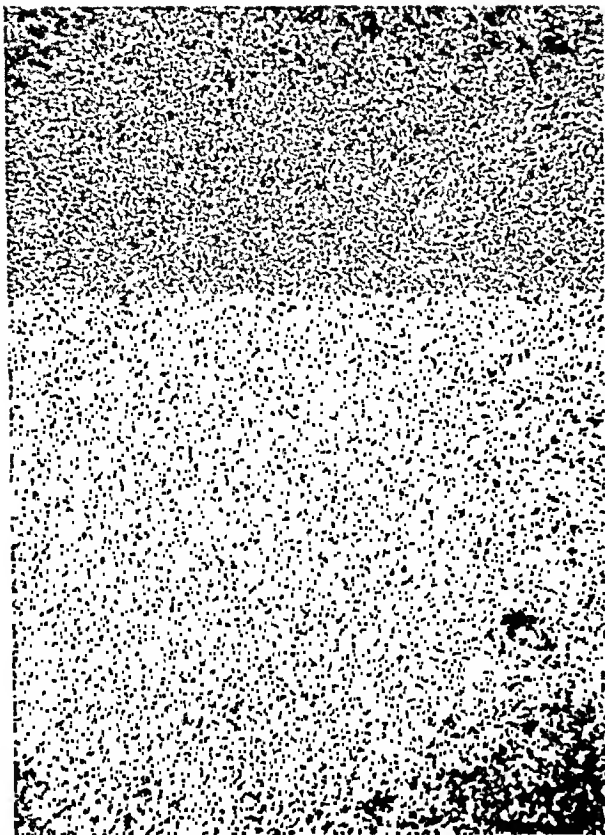
PLATE 18

FIG. 15. A marked increase of reticulum around an arteriole of the spleen from case 2 is shown by Foot's reticulum stain. ✕ 200.

FIG. 16. Section of the spleen from case 4, showing a widely patent sinusoid lined with swollen endothelial cells. Some of these cells have been extruded into the lumen as monocytes. Hematoxylin and eosin stain. ✕ 400.
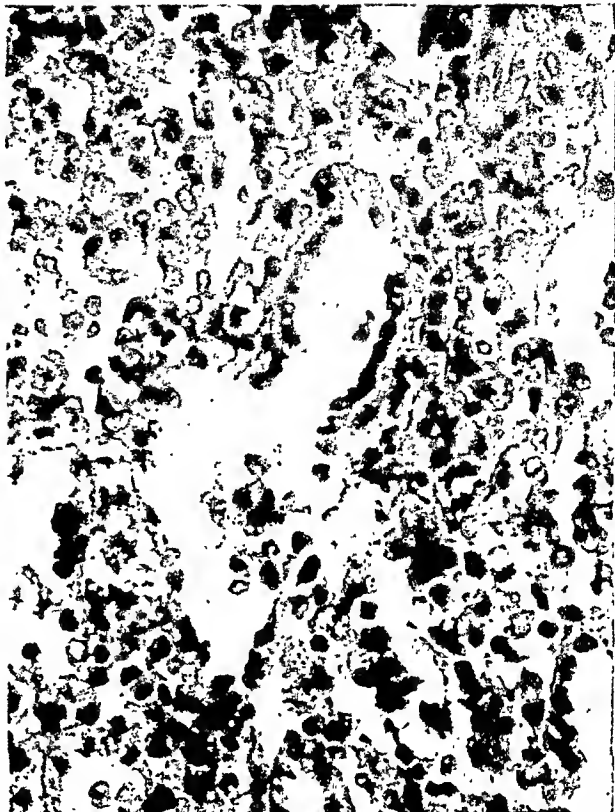
FIG. 17. Another section of the spleen from case 4. showing an intimate relation between the reticulum and monocytes. Hematoxylin and eosin stain. ✕ 400.

FIG. 18. The liver from case 2 shows a heavy infiltration of the portal areas with leukemic cells. Hematoxylin and eosin stain. ✕ 50.

5

16

17

18
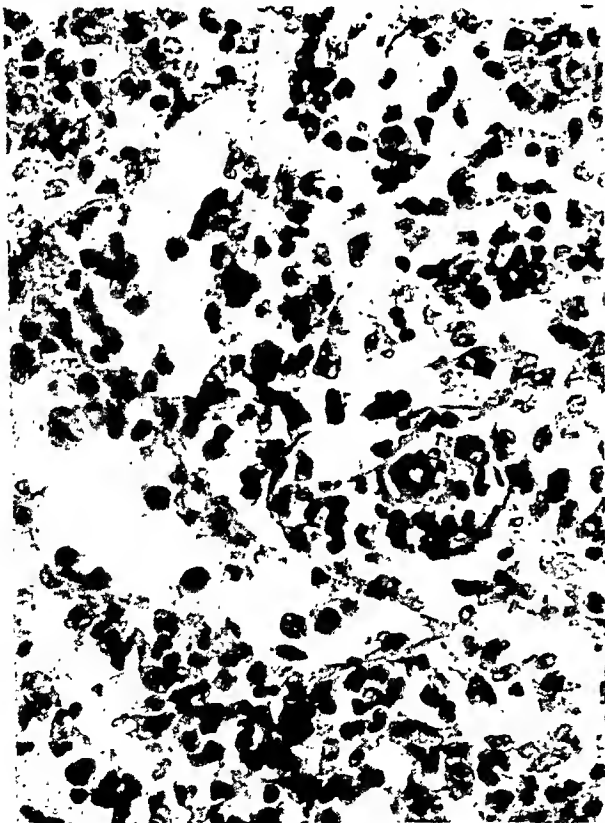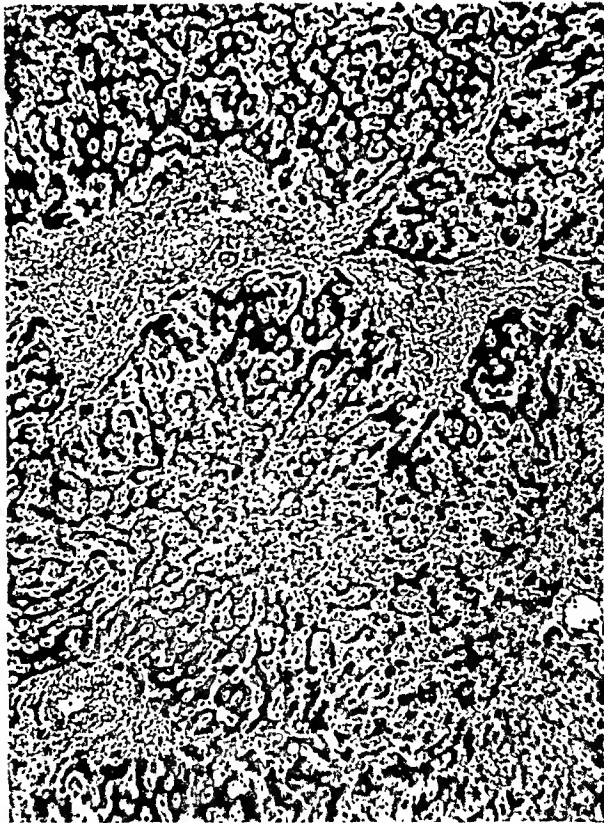
FIG. 19. Section of the liver from case 1, showing swelling of the sinus endothelial cells with extrusion of some of the cells into the lumen. One of the extruded cells in the center of the photomicrograph is in mitosis. Hematoxylin and eosin stain. $\times$ 400.

FIG. 20. Another area of the liver from case 1 to show Kupffer's cells in various stages of swelling and detachment into the lumina. Hematoxylin and eosin stain. $\times$ 400.

FIG. 21. Section of the liver from case 7, showing leukemic infiltration between the hepatic cords and its absence in the portal areas. Hematoxylin and eosin stain. $\times$ 50.

FIG. 22. The vertebral bone marrow from case 1 shows an almost complete replacement of the normal cells by monocytes, with aggregation of the latter around a blood vessel. Hematoxylin and eosin stain. $\times$ 50.
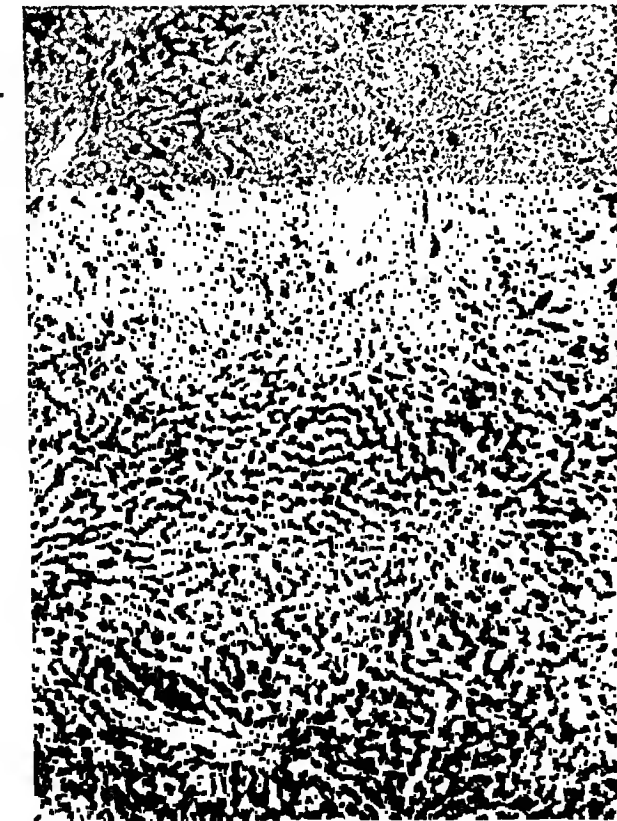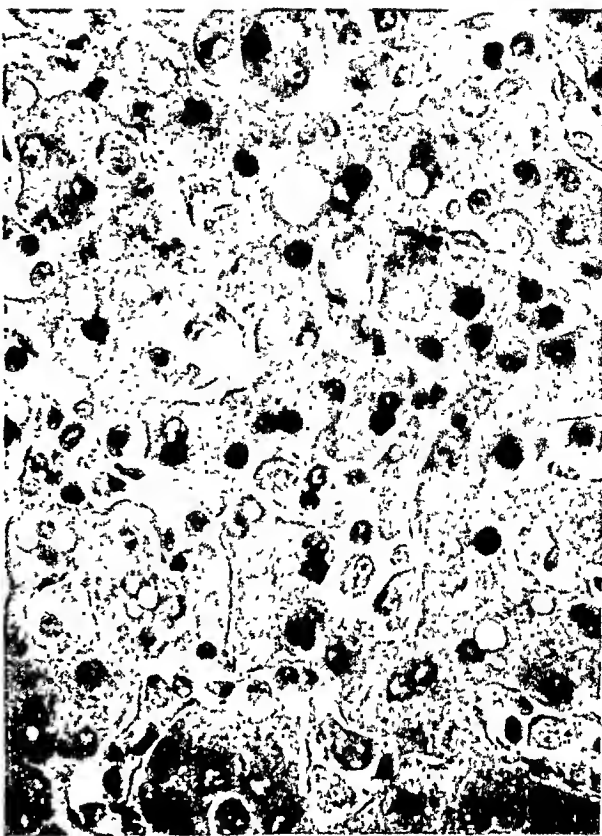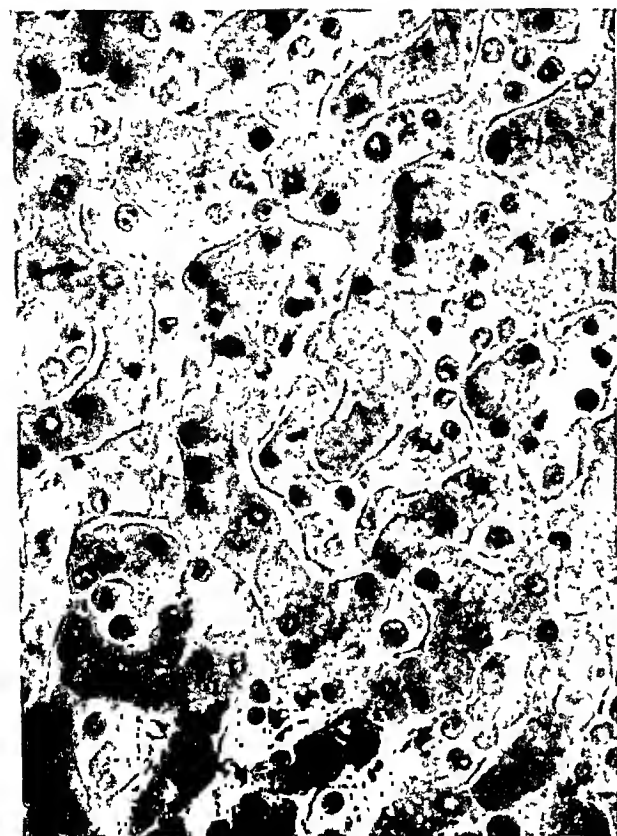
FIG. 23. Section of sternal bone marrow from case 7, showing a complete replacement of the normal cells with monocytes. Hematoxylin and eosin stain. $\times$ 50.

FIG. 24. The sternal marrow from case 8 shows huge monocytes, some of which have thin processes attached to the intermingled reticulum. Hematoxylin and eosin stain. $\times$ 400.

FIG. 25. Section of a lymph node from case 4, showing marked hyperplasia of the sinus reticulum with crowding of the lymphoid elements. Hematoxylin and eosin stain. $\times$ 75.

FIG. 26. A lymph node from case 7 shows complete replacement of the normal architecture by monocytes and an infiltration of the capsule by similar cells. Hematoxylin and eosin stain. $\times$ 50.
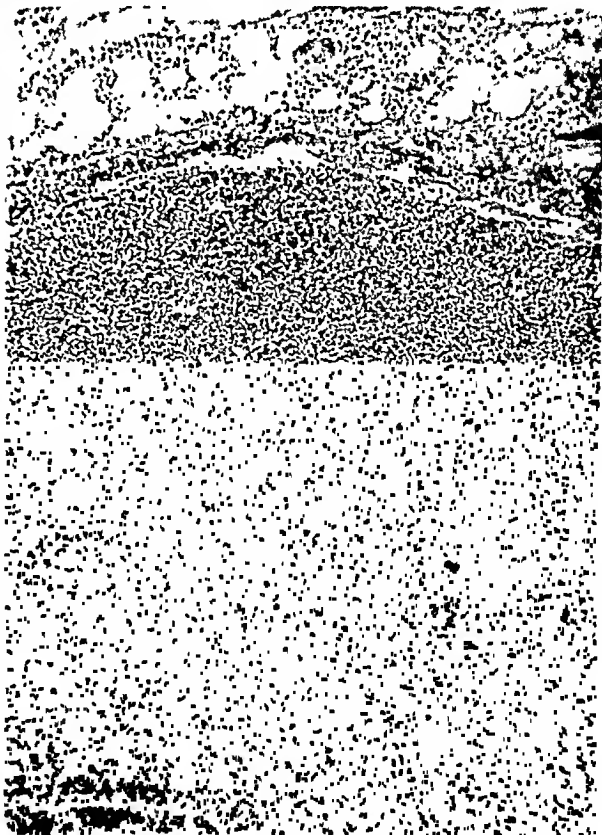
FIG. 27. Section of a lymph node from case 7, showing an area of reticulum hyperplasia. Foot's reticulum stain. × 200.

FIG. 28. Section of a lymph node from case 2, showing a picture resembling Hodgkin's disease. Large cells of the Sternberg-Reed type, eosinophils, lymphocytes, and a few polymorphonuclear leukocytes are present. Giemsa's stain. × 200.

FIG. 29. This area from a lymph node from case 1 shows at the lower right marked reticulum cell hyperplasia of the medulla with some maturation into monocytes. At one point the thin capsule is broken and there appears to be an outpouring of somewhat more mature monocytes into the cortex. Hematoxylin and eosin stain. × 200.

FIG. 30. Section of a lymph node from case 8, showing a picture somewhat similar to a "sarcomatous" type of Hodgkin's disease. The giant cells are larger, have more cytoplasm and more irregular borders than do those illustrated in Figures 13 and 28. Phagocytes, plasma cells, lymphocytes, and polymorphonuclear leukocytes are also present. Throughout this section there is a slight increase of the associated reticulum. Hematoxylin and eosin stain. × 200.
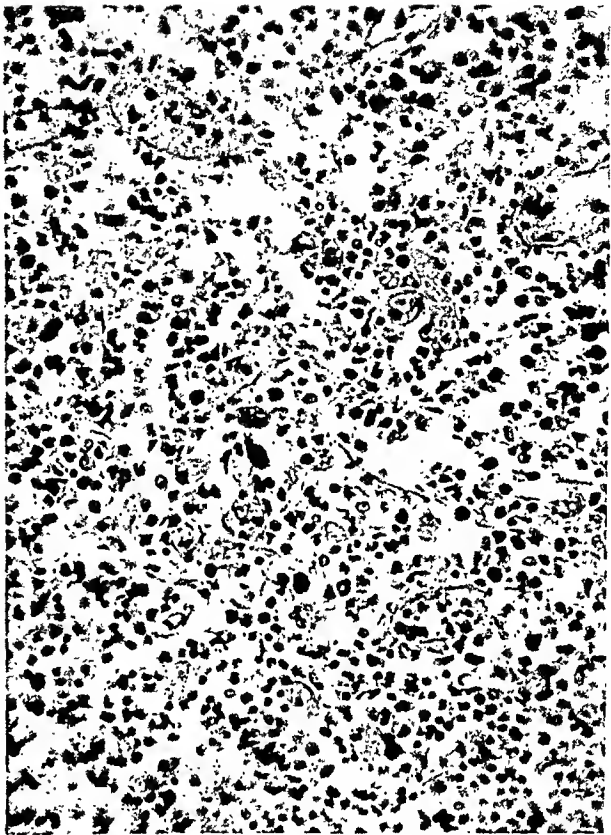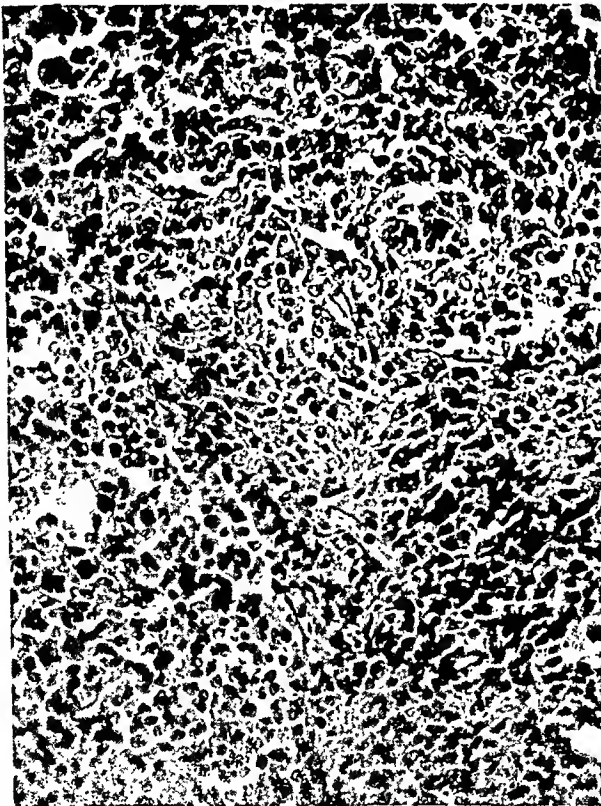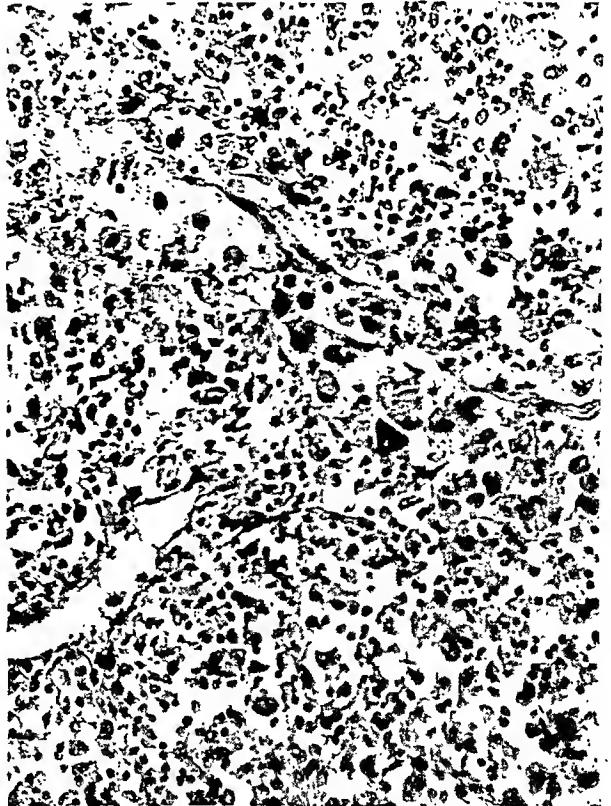
PLATE 21

PLATE 22

FIG. 31. Section of the thymus from case 1. showing a diffuse increase of reticulum in areas with reticulum cell hyperplasia, but a paucity of reticulum in areas containing mature monocytes (upper left corner). Foot's reticulum stain. × 200.

FIG. 32. Section of a germinal center in the small intestine from case 1, showing, in the lower portion, a marked reticulum cell hyperplasia with little maturation into monocytes, and a peripheral crowding of the remaining lymphocytes. Hematoxylin and eosin stain. × 200.

FIG. 33. In a kidney from case 6 there is a heavy interstitial infiltration with monocytes with partial obliteration of the renal parenchyma. Hematoxylin and eosin stain. × 50.

FIG. 34. Section of a vessel in the brain from case 2, showing an adventitial proliferation of reticulum cells and their maturation into monocytes. Hematoxylin and eosin stain. × 400.

31
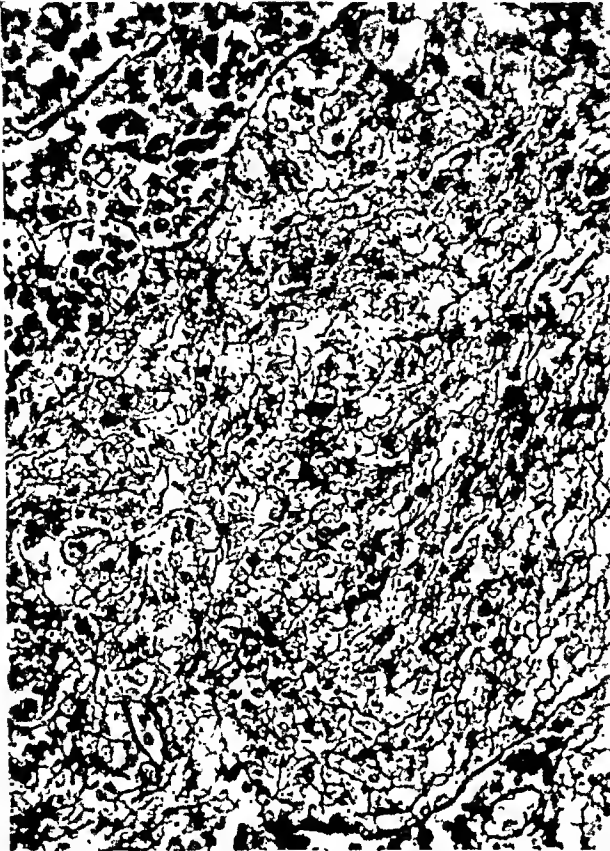
32
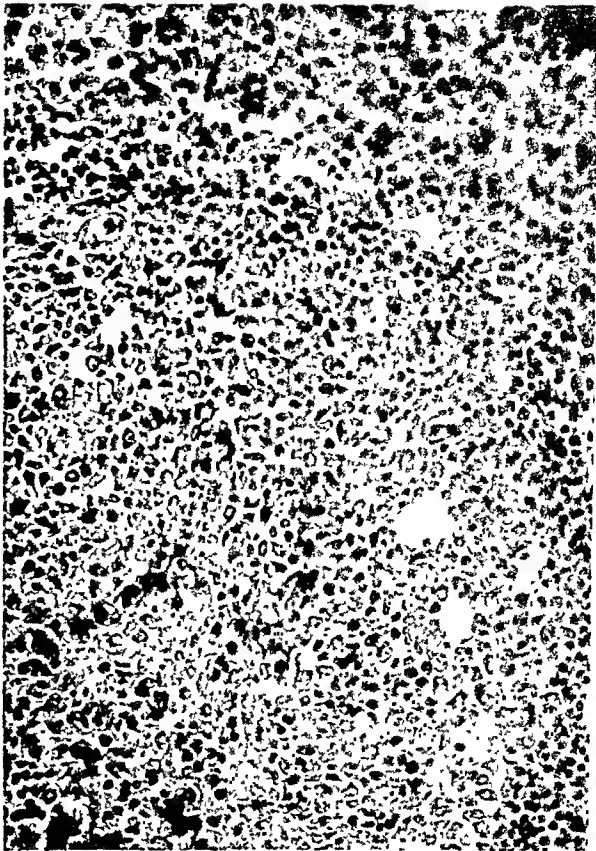
33

34
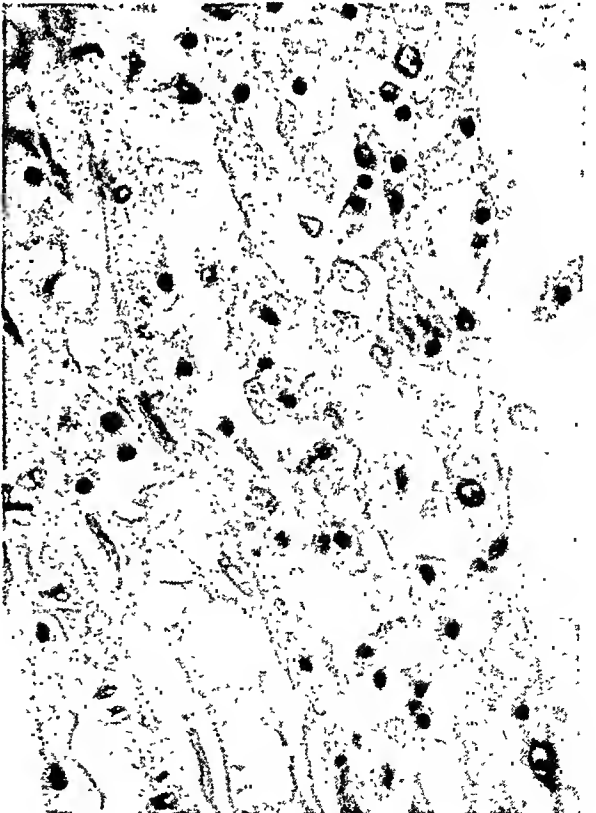
DRAINAGE OF THE PULMONARY VEINS INTO THE DUCTUS VENOSUS ARANTII

REPORT OF A CASE *

W. Harrison Mehn, M.D., and F. E. Hirsch, M.D.

(From the Departments of Pathology of the Illinois Masonic and Children's Memorial Hospitals and the Otho S. A. Sprague Memorial Institute Laboratory, Chicago, Ill.)

Anomalous drainage of the pulmonary veins has been reported frequently in the literature since the first case of this type was described by Winslow[1] in 1739. In analyzing the literature up to 1942, Brody[2] classified the examples of anomalous pulmonary drainage into two groups: cases in which only a part of the blood from the lungs entered the right atrium by direct or indirect routes; and cases in which all of the pulmonary blood reached the right atrium. In the latter group of 23 cases, the course of the blood from lungs to right atrium was, in order of frequency, by way of anomalous vessels to the superior vena cava or its tributaries, to the coronary sinus, directly to the right atrium, or to the portal vein or the inferior vena cava.

Drainage of all of the blood from the lungs into the right atrium, either directly or indirectly, is believed to be incompatible with life. Brody[2] stated that apparently a minimum of 50 per cent of the aerated blood must reach the left atrium to sustain life. Compere and Forsyth[3] estimated that when as much as 75 per cent of the pulmonary blood empties into the left atrium there will be no recognizable cardio-respiratory symptoms.

REPORT OF CASE

The following is a case of congenital malformation of the heart and aorta associated with drainage of the pulmonary veins into the ductus venosus.

B. F., a white male infant, was born on January 23, 1945, following a normal pregnancy. The birth weight was 10 lbs., 1½ oz. At the time of birth the child was cyanotic and had the umbilical cord wrapped twice about his neck. In other respects the delivery was uncomplicated. After stimulation the child began to breathe and the color improved. Respirations were regular and the infant's color continued to improve. At the age of 17 hours no abnormalities were noted. At 30 hours the infant suddenly became dyspneic and cyanotic with slight retractions of the right side of the chest. The heart was thought to be enlarged bilaterally. Râles were audible in both lung fields. No heart murmurs were heard. The rectal temperature was 103° F. Subcutaneous fluids were given and the child was placed in an oxygen tent. In spite of oxygen cyanosis persisted. On the following day an immense cardiac dilatation to the left was noted, but no murmurs were heard. A diagnosis of a congenital defect of the heart was made. The infant was then taking and retaining formula feedings up to 1½ oz. and was restful and quiet

with oxygen. On the fourth day a prominent precordial systolic murmur was heard. This murmur persisted until the seventh day of life, after which it could no longer be heard. On the twelfth day the infant suddenly became very dyspneic, more deeply cyanotic, and expired after 45 minutes of severe respiratory distress.

Post-mortem examination revealed a cyanotic infant in whom all organs were normal except for anomalies of the heart, aorta and pulmonary veins. The superior and inferior venae cavae emptied into a right atrium which was normal except for a widely patent foramen ovale with a diameter of 13 mm. The right ventricle was greatly increased in size and its musculature was 5 mm. in thickness. Tricuspid and pulmonary valves were enlarged to a circumference of 43 and 33 mm., respectively, but had normal cusps. At the point of bifurcation of the pulmonary artery (diameter, 8 mm.) a patent ductus botalli, 7 mm. in length and 5 mm. in diameter, extended to the arch of the aorta. The left side of the heart was very small. No blood vessels entered the left atrium. The left ventricular musculature had a thickness of 3.5 mm. Mitral and aortic valves were normal in shape and each had a circumference of 22 mm. In the interventricular septum at a distance of 5 mm. from the apex of the heart was a small defect, 2 mm. in diameter (Fig. 1). The aorta, slightly increased in size (diameter, 9 mm.), had a normal configuration up to a point just distal to the left subclavian artery and just proximal to the point of entrance of the patent ductus botalli where its lumen was less than half its proximal size (diameter, 4 mm.) (Fig. 1 and Text-Fig. 1). The thoracic aorta distal to this coarctation was normal. The right pulmonary veins united near the hilus of the lung to form a common trunk. The veins from the left lung joined to form a common vessel in a similar manner. These two common trunks fused in the midline posterior to the heart (Fig. 2 and Text-Fig. 1) to form a single vessel, with a diameter of 6 mm., which descended from this junction anteriorly to the esophagus and aorta, and passed through the diaphragm in close proximity to the inferior vena cava to empty into the ductus venosus at a point 2 mm. above the obliterating umbilical vein. During its course downward this vessel decreased gradually in size so that at the level of the ductus venosus it had a diameter of only 2 mm. The ductus venosus was still patent with a diameter of 4 mm. It communicated freely with the inferior vena cava through a short trunk 8 mm. in length and 3 mm. in diameter (Fig. 2 and Text-Fig. 1).

Blood, oxygenated in the lungs, passed through the common trunk formed by the pulmonary veins into the ductus venosus arantii and thence into the inferior vena cava, with a probable backflow into the distal portion of the obliterating umbilical vein and the adjacent por-

tal vein. Oxygenated blood was thus mixed with the purely venous blood of the inferior vena cava. As it entered the right atrium the blood was again mixed with purely venous blood of the superior vena cava. From the right atrium the mixed blood passed into the pulmonary circuit, through the patent ductus botalli into the peripheral circulation, through the open foramen ovale into the small left atrium, and through the small interventricular defect into the left ventricle. The mixed blood from the left atrium and left ventricle passed into the aortic arch and
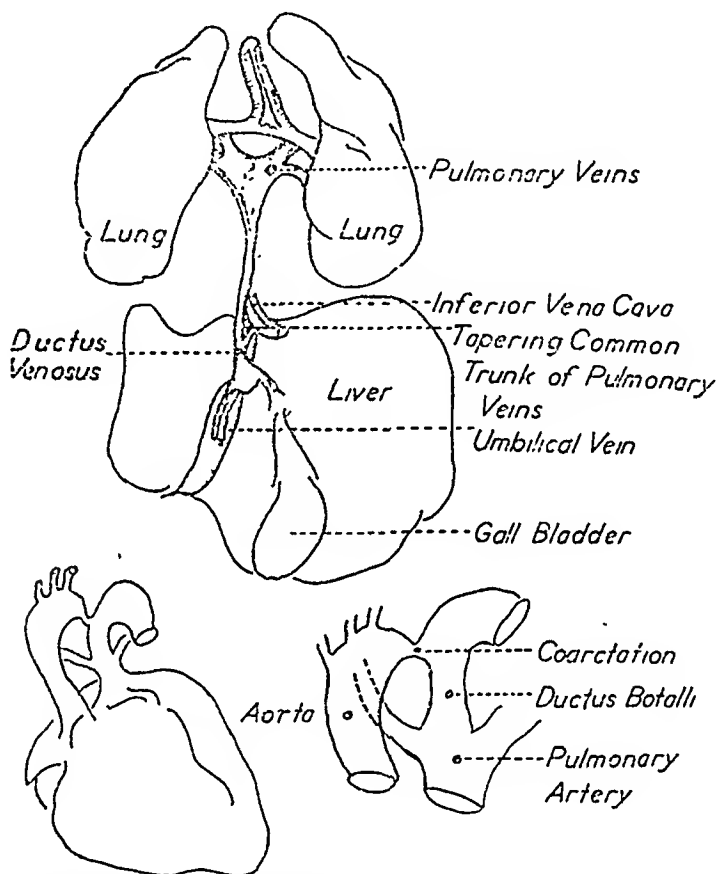
Text-Fig. 1. Diagrammatic representation of the autopsy specimen. Of note is the configuration of the aorta and pulmonary artery in the lower right corner.

its vessels, encountering resistance at the point of coarctation. No organ or part of any organ received purely arterial blood.

DISCUSSION

A search through the literature revealed only one case of a somewhat similar anomalous pulmonary drainage (Table I). Ghon,[4] in 1916, described a 15-day-old infant in whom the pulmonary veins of each side united to form a common stem. These, in turn, formed another venous stem, 1 cm. in circumference, which lay anterior to the esophagus and aorta, proceeded inferiorly to pierce the diaphragm with the inferior vena cava and opened into a vessel which joined the still

patent ductus venosus arantii. Blood, arterialized in the lungs, passed by this route into the first part of the ductus venosus arantii and thence into the sinus of the left branch of the portal vein. The foramen ovale and ductus botalli were still patent.

Several cases have been reported in which the pulmonary drainage completely or incompletely emptied into blood vessels inferior in position to the right atrium. In such cases the embryological explanation of the anomalous drainage is probably similar to any which might be advanced for the case here reported.

TABLE I

Reported Cases

Author	Sex	Age	Drainage
Arnold[5]	F	15 weeks	Portal vein
Bochdalek[6]	M	4 days	Right upper, into superior vena cava; remainder of pulmonary veins into portal vein
Chassinat[7]	F	12 days	Right, into inferior vena cava
Cooper[8]		10 months	Right, into inferior vena cava
Geipel[9] (Right, half of thoracopagus)	F	5-6 month of pregnancy	Portal vein
Ghon[4]	M	15 days	Portal vein
Hu[10]	M	7 months	Portal vein (complete drainage of both lungs)
Munck[11]	M	3 months	Portal vein
Park[12]*	M	2½ months	Right, into inferior vena cava
Ramsbotham[13]		Newborn	Left, into subclavian vein; right, into portal vein
Terplan and Sanes[14]			Portal vein
Uchida[15]		Fetus	Inferior vena cava

* Case also reported by Brown.[16]

Zuckerkandl [17] (1881), by injections through the pulmonary vein, found that the injection mass filled not only the pulmonary veins themselves but also the bronchial veins and through them the venae azygos and hemi-azygos, and the mediastinal network of veins and, in many instances, reached even the postcaval and gastric veins. The pulmonary veins were seen to anastomose regularly with the veins of the mediastinal network. He concluded that anomalies might be caused by overgrowth or underdevelopment of capillary plexuses at a place where the vein in question should develop. He reasoned that pulmonary and systemic veins might merely be remnants of an original indifferent plexus from which drainage is developed to best suit the organ or tissue.

Evans,[18] in discussing theories of vascular systems, stated that vessels may develop secondarily in their own region from capillary plexuses. Blood vessels, according to this theory, develop in response to the need and the function to be performed by the organ in which the vascular system is found. The assumption that tissues and organs have a

rôle of importance in respect to plexuses and vessels may, he believed, be of assistance in explaining vascular patterns.

The present knowledge of the origin of the pulmonary veins is incorporated in two somewhat opposing views. One holds that the veins develop as a proliferation from the dorsal endothelial wall of the sinus venosus toward the lung buds, and is supported by investigators such as Federow,[19] Flint,[20] and Buell.[21] The other view is that the veins develop from communications between the sinus venosus and an indifferent plexus originally present in the region, and is supported by Brown[16] and von Möllendorff.[22]

In the view held by Brown,[16] no explanation is given of the source of the plexus, but many agree that the plexus about the lung bud is not specific, having been developed from the splanchnic plexus of the foregut. The conception that the pulmonary system is only a specially developed part of an indifferent plexus serves to explain communications between pulmonic and systemic circulations which are normally found and also those which constitute severe anomalies of the pulmonary veins. For example, an anastomosis (MacCready[23]) between the splanchnic plexus and the omphalomesenteric veins is a probable cause of a communication between the pulmonary and portal systems. Persistence of connection between pulmonary veins and the esophageal plexus and its connection with the developing inferior vena cava would account for a condition in which pulmonary veins open into the inferior vena cava.

SUMMARY

In the case reported, pulmonary venous drainage was into the ductus venosus arantii. Only one somewhat similar case was found in the literature although several cases have been reported with complete or incomplete pulmonary venous drainage into vessels inferior to the right atrium.

REFERENCES

1. Winslow, M. (Cited by Compere and Forsyth.[3])
2. Brody, H. Drainage of the pulmonary veins into the right side of the heart. *Arch. Path.*, 1942, 33, 221–240.
3. Compere, D. E., and Forsyth, H. F. Anomalous pulmonary veins: report of a case. *J. Thoracic Surg.*, 1944, 13, 63–66.
4. Ghon, A. Ein Beitrag zu den Anomalien der Pulmonalvenen. *Beitr. z. path. Anat. u. z. allg. Path.*, 1916, 62, 175–193.
5. Arnold, J. Ein Fall von Cor triloculare biatriatum, Communication der Lungenvenen mit der Pfortader und Mangel der Milz. *Virchows Arch. f. path. Anat.*, 1868, 42, 449–472.
6. Bochdalek. Beschreibung einer sehr merkwürdigen Abweichung der Lungenvenen bei einem 4 Tage alten an Peritonitis verstorbenen Knaben. *Vrtljschr. f. d. prakt. Heilk.*, Prag., 1858, 60, 160–174.

7. Chassinat, R. Observation d'anomalies anatomiques remarquables de l'appareil circulatoire, avec hépatocèle congéniale, n'ayant donné lieu pendant la vie à aucun symptôme particulier. *Arch. gén. de méd.*, 1836, 11, 80–91.

8. Cooper, G. Case of malformation of the thoracic viscera, consisting of imperfect development of right lung, and transposition of the heart. *Lond. M. Gaz.*, 1836, 18, 600-602.

9. Geipel, P. Ein Beitrag zur Lehre des Situs transversus. Festschrift zur Feier des fünfzigjährigen Bestehens des Stadtkrankenhauses zu Dresden-Friedrichstadt, 1899, pp. 373–436.

10. Hu, C. H. Congenital malformation of the heart with anomalous insertion of pulmonary veins, absence of spleen, situs inversus of abdominal viscera and other developmental errors. *Am. J. Path.*, 1929, 5, 389–396.

11. Munck, W. Über abnormen Verlauf der Lungenvenen. *Acta path. et microbiol. Scandinav.*, 1933, 10, 321–329.

12. Park, E. A. Defective development of the right lung, due to anomalous development of the right pulmonary artery and vein, accompanied by dislocation of the heart simulating dextrocardia. *Proc. New York Path. Soc.*, 1912, 12, 88–93.

13. Ramsbotham, F. (Cited by Gohn.[4])

14. Terplan, K., and Sanes, S. The incidence of congenital heart lesions in infancy; a comparative statistical study based on post-mortem examinations. *J. Tech. Methods*, 1936, 15, 86–95.

15. Uchida, S. Case of situs inversus. *Jap. J. Obstet. & Gynec.*, 1928, 11, 64.

16. Brown, A. J. The development of the pulmonary vein in the domestic cat. *Anat. Rec.*, 1913, 7, 299–329.

17. Zuckerkandl, E. Über die Anastomosen der Venae pulmonales mit den Bronchialvenen und mit dem mediastinalen Venennetze. *Sitzungsb. d. k. Akad. d. Wissensch. Math.-naturw. Cl.*, 1881, 84, Pt. 3, 110–152.

18. Evans, H. M. Die Entwicklung des Blutgefässsystems. In: Keibel, F., and Mall, F. P. (eds.). Handbuch der Entwicklungsgeschichte des Menschen. S. Hirzel, Leipzig, 1911, 2, 551–688.

19. Federow, V. Ueber die Entwicklung der Lungenvene. *Anat. Anz.*, 1908, 32, 544–548.

20. Flint, J. M. The development of the lungs. *Am. J. Anat.*, 1906–07, 6, 1–137.

21. Buell, C. E., Jr. Origin of the pulmonary vessels in the chick. *Contributions to Embryology, no. 66*, Carnegie Institute of Washington, 1922, 14.

22. von Möllendorff, W. Über Anlage und Ausbildung des Kiemenlungenkreislaufs bei Anuren (Bombinator pachypus). *Anat. Hefte*, 1913, 47, 249–274.

23. MacCready, P. B. Anomalies of the pulmonary veins. *Bull. Johns Hopkins Hosp.*, 1918, 29, 271–275.

DESCRIPTION OF PLATE

PLATE 23

FIG. 1. Drawing of the heart as seen from the left, with the flap of the left ventricle removed and the left atrium reflected. S.V.C. = opening of superior vena cava; S.D. = location of atrial septal defect; I.V.C. = opening of inferior vena cava; V.S.D. = ventricular septal defect. Because of the almost complete absence of atrial septum, the view is distorted; the venae cavae actually empty into the right atrium.

FIG. 2. Autopsy specimen, posterior view, with the flap of the left ventricle reflected. String (small arrow on left) passes through the ventricular septal defect.

Mehn and Hirsch   Anomalous Pulmonary Veins
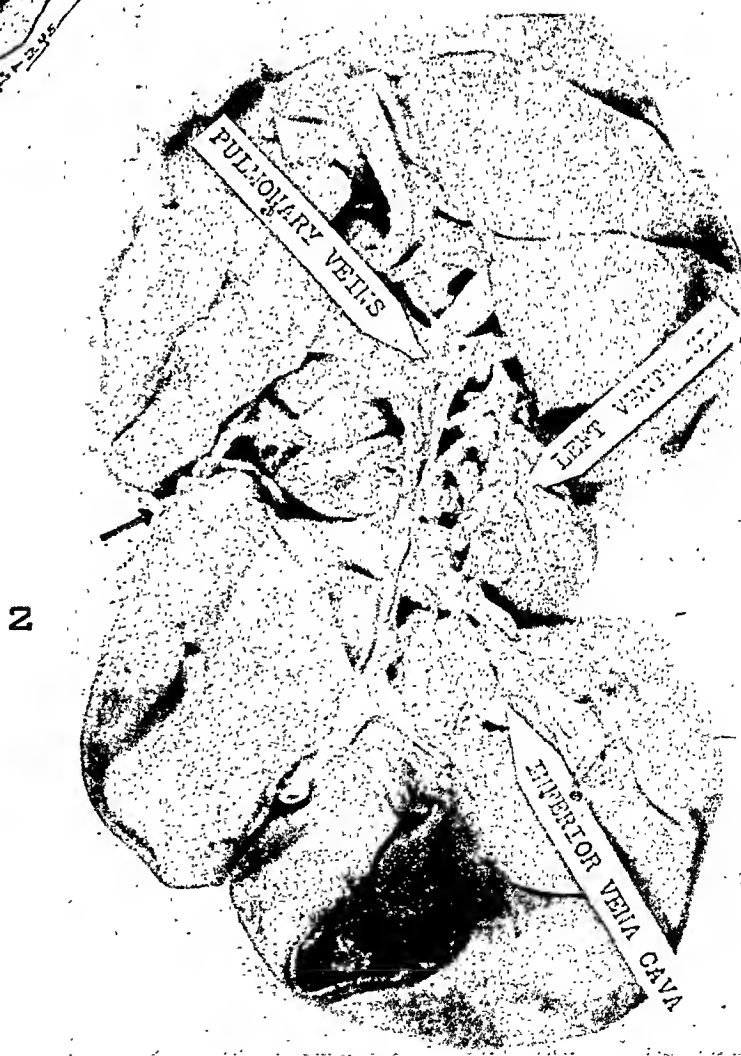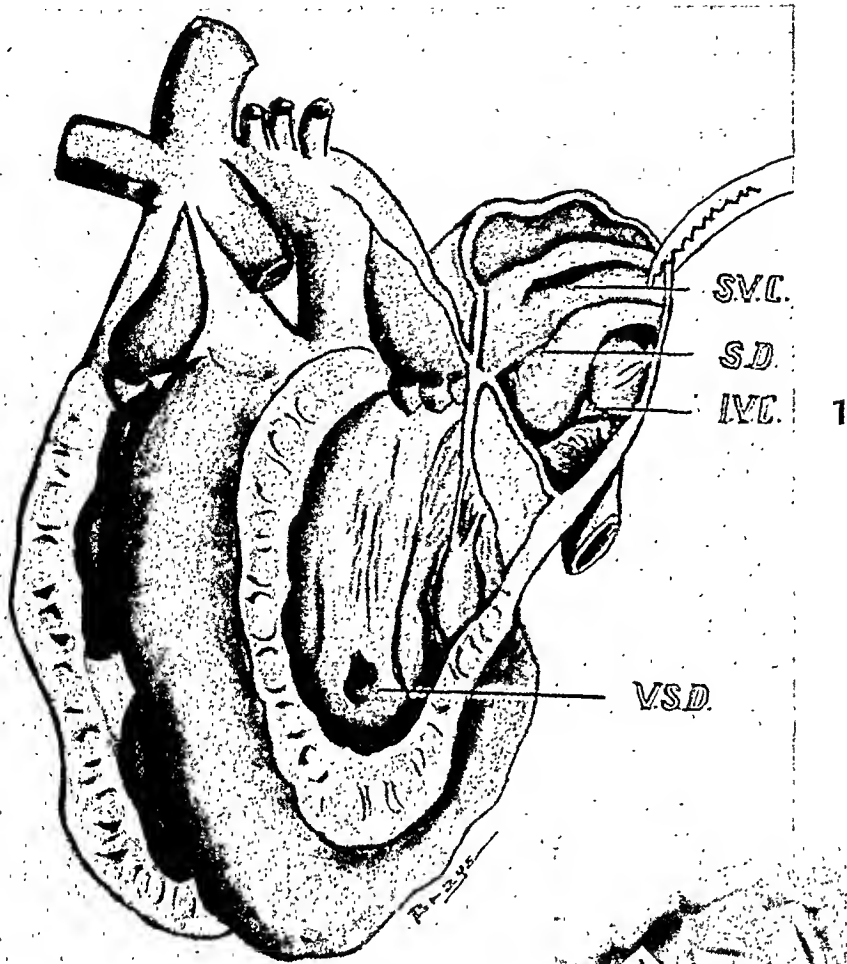
GROSS CEREBRAL HEMORRHAGE AND VASCULAR LESIONS IN ACUTE TUBERCULOUS MENINGITIS AND MENINGO-ENCEPHALITIS *

Joseph W. Goldzieher, M.D., and James R. Lisa, M.D.
(*From the Laboratory of Pathology, City Hospital, Welfare Island, Department of Hospitals, New York 17, N. Y.*)

Much has been written during the past few years about the pathogenesis of tuberculous meningitis and many of the older concepts have been subjected to critical review. The literature dealing with its morphologic pathology, on the other hand, has been extremely scanty during the past 15 years. In fact, the first article in the American literature stressing the vascular changes was that of Winkelman and Moore [1] in 1940.

The older textbooks [2-4] are in general agreement that hemorrhages ("red malacia") are a common finding in tuberculous meningitis. Jakob,[5] for example, stated that not uncommonly there may be large extravasations of blood, hemorrhagic malacia, or a purpuric appearance. Statements to this effect are still to be found in recent textbooks,[6] although the experience of a number of present-day pathologists [7,8] does not confirm this impression. Stevenson [9] has stated that hemorrhages in tuberculous meningitis have become as rare as the conglomerate tubercle or the gumma.

The available literature shows a wide variation in the reported incidence of hemorrhagic phenomena. Hayem,[10] in 1868, described a case of hemorrhagic tuberculous encephalitis. Hoche,[11] in 1888, associated venous obliteration with isolated hemorrhages in one case. Gavazzeni [12] reported 2 cases in 1901. In 1902 Bombicci [13] reported 5 cases in infants or young children in the course of a lengthy study of hemorrhagic encephalitis. He noted that hemorrhages were either peripheral, usually frontal, or in the basal ganglia, and ranged up to 3 cm. in diameter. The hemorrhagic phenomena were ascribed chiefly to venous thrombosis due either to endothelial changes or "toxic factors." Aneurysmal dilatations were observed in one of the cases. Single cases were published by Vanzetti [14] in 1904 and by Grafe and Gross [15] in 1920. In the classic paper of Askanazy [16] in which the hyaline-fibrinoid degeneration now known by his name was first described, hemorrhages were found in 3 of 23 cases and were considered a rare phenomenon. Kirschbaum [17] mentioned hemorrhages in 5 cases. Winkelman and Moore [1] described no hemorrhages in the 5 cases which they presented in detail and did not mention their presence in the total of some 200 cases studied. It is also noteworthy that the Askanazy degeneration

was not seen in their series. MacGregor, Kirkpatrick, and Craig[18] (1935) made no mention of hemorrhages. Biber[19] is the sole observer who found hemorrhages to be frequent: he demonstrated them in 15 of 17 cases.

Within the past year there have been autopsied at City Hospital, Welfare Island, 2 cases of acute tuberculous meningo-encephalitis which had gross hemorrhages and extensive vascular lesions of an unusual nature. They form the basis of this communication.

REPORT OF CASES

Case 1

The patient, S.R. (no. 132792), was a Negress, 36 years old, who was admitted to the First Medical Division, service of Dr. J. Homer Cudmore, on April 1, 1945. Her chief complaint was severe headache of 8 days' duration. She believed that she had had some fever for the past month. There had been photophobia and a slight cough.

Examination revealed semistupor, moderate tenderness of the left lower quadrant of the abdomen, general hyperreflexia, the right knee jerk more active than the left, a positive Kernig sign, and a cross extensor reflex. The spinal fluid pressure was 370 mm. of water; cells, 450 per cmm. of which 60 per cent were small lymphocytes; sugar, 27 mg. per cent; protein, 62 mg. per cent; chlorides, 737 mg. per cent; Wassermann reaction, negative; gold-sol curve, 123443210; Levinson and tryptophane tests, positive. Culture yielded no growth. The white cell count was 9500; polynuclear cells, 68 per cent; lymphocytes, 26 per cent; eosinophils, 4 per cent; monocytes, 2 per cent.

The course was characterized by continuous low-grade fever, bradycardia, and progressive neurologic signs. The mental status showed a transient improvement but nuchal rigidity remained and nystagmus to the left was noted. Gradually the patient became apathetic, then irrational. Three days before death there were oculomotor paralysis and dilatation of the right pupil. Death occurred in coma on the 15th hospital day.

Examination of the spinal fluid obtained during the course of the illness showed a fall of the sugar to 18 mg. per cent and the chlorides to 684 mg. per cent. The Levinson and tryptophane tests remained positive. Cultures were sterile; a pellicle formed in one specimen.

Autopsy (no. 7221) was performed after the body had been embalmed. Only the findings for the brain are given in detail.

The entire base of the brain was covered by a thick, dense, yellow, fibrinous exudate which obscured the cranial nerves. There were numerous firm, stringy adhesions between the dura and leptomeninges of the frontal lobes. Over the surface were many minute, tubercle-like nodules, especially marked along the sulci. On sectioning, a small area of petechial hemorrhage was found just superior to the right inferior frontal gyrus and another on the antero-inferior aspect of the cerebellum. The area bordering the fourth ventricle was diffusely reddened by confluent petechiae (Fig. 1). There were a few more in the left putamen. The walls of the lateral ventricles had a finely granular

appearance, more extensive on the floor and extending upwards. The right ventricle was more affected than the left.

Microscopically, the meninges showed an extensive inflammatory reaction which varied from place to place. In the vicinity of some blood vessels there was intense serous exudation with only scanty cellular infiltration. In other areas large patches of fibrin were present. Some small areas showed an almost pure polynuclear infiltration, although a pleomorphic cellular response was far more common, usually consisting of polynuclear cells, small lymphocytes, plasma cells, phagocytes, swollen epithelioid cells, and débris. Frequently, small foci of incipient necrosis were found. Cellular débris was found in the center of nodules of epithelioid cells; in these areas tubercle bacilli were demonstrable. Only rarely were giant cells or characteristic tubercles to be found.

The meningeal vessels were extensively involved in this process. Intense perivascular infiltration was found everywhere. The adventitia was infiltrated by lymphocytes, plasma cells, and phagocytes. Occasionally the muscularis of the arteries showed disorganization of the architecture with coagulation necrosis or rhexis of the muscle fibers and infiltration with wandering cells or young fibroblast-like cells. In such areas the elastic fibers were either fibrillated, completely destroyed, or stained very poorly. Frequently the elastica interna was frayed, poorly stained, or completely torn, and the cellular infiltrate seemed to pour through this break and spread fanwise beneath the endothelium (Fig. 2). Incipient aneurysms were seen elsewhere (Figs. 3 and 4). Acid-fast bacilli were found in the adventitia or media of the affected areas, but none were found in the subintimal infiltrate. One subintimal tubercle with a giant cell was found, and within a single endothelial cell of an intact arteriole a juxtanuclear tubercle bacillus was seen. The subintimal infiltrate varied in character. In the least affected areas there might be only a single layer of cells, chiefly lymphocytes and occasionally polynuclear cells. In more severely involved regions macrophages and fibroblast-like cells also were found. No red cells or histiocytes were seen. Of great interest was the hyaline-fibrinoid degeneration of media and intima which was found in the smaller arteries and even in the smallest arterioles. The larger vessels (1.5 mm. in diameter and larger) showed pronounced changes of the elastica interna, but hyaline material was found in the intimal zone with great rarity. Almost all of the veins showed destruction of the elastic fibers; this was not constant in the arteries. The arterioles seemed to be more affected by the hyaline degeneration than the larger vessels. In many areas nearly every arteriole showed a dense, smudgy material

in the subintimal zone (Fig. 5); frequently there was thrombosis within the lumina. Occasionally a perivascular cellular infiltrate extended down and around these vessels, and this might be found even in the vicinity of capillaries (Fig. 6). An occasional artery was thickly mantled by large, uniform macrophages (Fig. 7). In the cerebellar tissue, particularly, destruction of the vascular walls was prominent; numerous thrombi were seen.

The brain tissue bordering on the meninges showed a narrow zone of infiltration in most areas. Gitter cells were observed and at times even the deeper tissues showed infiltrative and proliferative changes in areas where the inflammatory reaction extended deep into the sulci along the vessels. Acute edematous changes of a focal character also were present and there were large patches of proteinic exudate in the Purkinje layer of the cerebellum. In a few areas, characteristic tubercles of the cortex were found. The hemorrhages observed grossly were confined to those areas where vascular involvement and thrombosis were present. Sections from the frontal lobe showed hemorrhage around the vessels in Virchow-Robin's space and infiltrating the parenchyma in cuff-like fashion. In the cerebellum, vascular destruction was more marked, thrombi were common, and large areas of parenchyma were completely destroyed by hemorrhage.

The other pertinent anatomic findings were bilateral hydronephrosis, chronic cholecystitis, chronic salpingitis, and a Ghon tubercle of the left lower lobe of the lung. The diagnosis as to the brain was acute tuberculous meningitis and encephalitis.

Case 2

The patient, E.H. (no. 134514), a female cretin dwarf, 48 years old, was admitted to the First Medical Division, service of Dr. John Carroll, on July 25, 1945. The history was that of increasing stupor for 2 days, unaccompanied by pain, headache, or nausea. The only pertinent fact elicited in the past history was an attack of "flu" 4 months previously from which she "never fully recovered"; it had not been followed by residual cough or other respiratory symptomatology.

Physical examination revealed slight nuchal rigidity, positive Brudzinski sign, positive right Babinski reflex, and a negative Kernig sign. The spinal fluid was under a pressure of 310 mm. of water, very cloudy, and contained 800 cells per cmm., 95 per cent polynuclear cells, and 5 per cent lymphocytes. The protein was 251 mg. per cent; sugar, less than 5 mg. per cent; chlorides, 910 mg. per cent; Wassermann reaction, negative; gold-sol curve, 233455555; culture, sterile. The hemal leukocyte count was 10,000 per cmm., with 80 per cent polynuclear cells.

The temperature rose rapidly to 105° F. Eighteen hours after admission there were generalized tonic and clonic convulsions which could be controlled only by heavy sedation. Death occurred 20 hours after admission.

Autopsy (no. 7295) was performed 2 hours after death.

Examination of the brain showed a small amount of fine, fibrinous exudate over the base; otherwise there was little of note in the

meninges. The frontal lobes were extremely soft and had a bluish discoloration. On section, these lobes were almost completely destroyed and were converted into a soft hemorrhagic paste. At the periphery, the brain tissue was studded with innumerable hemorrhages ranging from pin-point to 3 mm. in diameter. The remainder of the cerebrum, midbrain, pons, medulla, and cerebellum showed nothing of note.

Microscopically, throughout the frontal lobes the intracerebral vessels showed extensive hyaline-fibrinoid degeneration (Fig. 8) involving nearly every vessel in the area. A large proportion of the vessels were thrombosed. Extensive hemorrhages surrounded most of these vessels and hematogenous pigment was present in the adjacent tissue. There was patchy, occasionally very dense, polynuclear infiltration around the affected vessels, and these cells also infiltrated the better preserved cerebral tissue nearby. There was also a concomitant infiltration with lymphocytes and gitter cells. In other parts of the brain no vascular changes were seen; there was no hemorrhage or infiltration with polynuclear cells, but lymphocytes and gitter cells were evident.

The leptomeninges showed only some slight edema in all regions examined. Only in those regions where the meningeal vessels were involved did more extensive changes occur. In those localized areas nearly all meningeal veins showed a hyaline-fibrinoid degeneration and, frequently, obliteration of the lumen by hyaline thrombi. The walls of the vessels sometimes were indistinguishable in such sections. Around the vessels and extending for a short distance into the leptomeninges there was a fairly dense exudate composed of fibrin, numerous multilobed polynuclear cells, occasional macrophages, and innumerable extravasated red cells. A few millimeters away from such an area the meninges were again apparently normal. The small arteries showed little change save for the infrequent areas of subintimal infiltration, chiefly with polynuclear and occasionally mononuclear cells. No giant cells or tubercles were seen anywhere, and only rarely did small clumps of epithelioid cells occur. The gray matter beneath these involved areas showed perivascular infiltration with round cells and numerous gitter cells, occasionally forming a layer just beneath the surface of the cortex. Even the white matter far from any meningeal lesion showed dense clusters of round cells about capillaries and small vessels. Acid-fast bacilli were found with extreme rarity.

<div align="center">DISCUSSION</div>

<div align="center">*Hemorrhages*</div>

Several theories have been proposed to explain the occurrence of hemorrhages in tuberculous meningo-encephalitis. Askanazy[16] believed that hemorrhages were due to venous thrombosis, as is com-

monly seen in hemorrhagic infarcts elsewhere. However, he thought that arterial occlusion might also be a causative factor. Biber,[19] on the other hand, believed that hemorrhage was due to diapedesis or to actual vessel rupture. The latter did not emphasize the rôle of thrombosis, possibly because none of the cases which he examined showed the fibrinoid degeneration which we now know may give rise to extensive thrombotic phenomena.

In our material it was quite evident that thrombosis with subsequent hemorrhagic infarction played the chief rôle. It was therefore impossible to decide whether ruptures of the small vessels were due to intrinsic damage to the vessel wall or whether they were part of the usual process of infarction with the wall giving way under the increased circulatory pressure. Although incipient aneurysms were seen in the walls of some of the larger arteries (Figs. 3 and 4), no actual rupture of such an area was found. Thrombosis was an outstanding feature of both cases. Thrombi were by far more common in the veins, and the hyaline (platelet) thrombi described by Biber [19] were seen frequently. In all areas where hemorrhages were present both arteries and veins showed multiple thrombi.

Vascular Changes

The acuteness of the lesions in both cases afforded an unusual opportunity to trace the evolution of the pathologic process. The attack on the vessels, always more destructive to veins than to arteries, clearly begins with an inflammatory reaction in the immediately periadventitial region. This observation confirms the experimental findings of Rich and McCordock [20] who, on injecting tubercle bacilli into the subarachnoid space, noticed that bacteria had a tendency to cluster around the vascular channels and attack them from without. This observation also modifies considerably the usual concept that the vessels are only incidentally involved in the meningeal inflammatory process.

In the very early stages of inflammation, as in case 2, the cellular response is chiefly polynuclear in character. Older lesions, as in case 1, approach more closely the typical cellular reaction of tuberculosis; i.e., lymphocytes, plasma cells, macrophages, and occasional epithelioid cells. Rarely, a vessel may be mantled by large macrophages of a very uniform appearance. The character of the infiltrate varies with the rate of invasion of the vessel wall. In a rapidly destructive process the changes we have just described are found; in the slowly invading lesions tubercles, and eventually caseation, may appear.

The media is clearly the most resistant portion of the vessel wall. Even with a completely destroyed adventitia and thickly infiltrated intima the media is apt to be surprisingly well preserved. Occasional

cells may be seen apparently migrating through the media. When changes do occur, first a hyalinization and a slight basophilia of the muscle fibers appear. Later, there may be rhexis or even complete necrosis.

The elastica interna reacts to the inflammatory process by becoming fibrillar and fraying. Occasionally there is complete rupture. Some authors even have observed the torn ends to curl inward toward the vessel lumen,[19] a detail not seen in our material. Nor were we able to see any complete arterial ruptures or arterio-arterial fistulae as described by Biber,[19] although beginning aneurysmal formation was found.

The origin of the intimal lesions is probably manifold. Most commonly, the early intimal lesion consists of a pleomorphic cellular infiltrate beginning as a single layer of cells similar to the type of infiltration seen around the vessel. We were able to observe several lesions in which the perivascular infiltrate extended down through the media and the frayed elastica interna, and then seemed to spread out fanwise as soon as it reached the intimal zone. The tapering off of the density of the infiltrate as well as the absence of proliferative changes, considering the size of the lesion, is strongly suggestive of this sequence of events. Involvement of the intimal zone by way of the blood stream, which would correspond to the "tuberculous endarteritis" of Kirschbaum,[17] is a possibility not to be ignored. The existence of isolated intimal tubercles, as first shown in serial sections by Hektoen,[21] is strong evidence to support this concept. Moreover, in our case 1 we observed a tubercle bacillus actually within the swollen endothelial cell of an otherwise intact arteriole. This finding seemingly lends support to the hematogenous origin of some of the intimal lesions.

The hyaline-fibrinoid degeneration of Askanazy[16] was present to a marked degree in both of our cases. The peculiar fibrin-like material could be found both in the subintimal zone and in the media (Figs. 5 and 8). The frayed elastica interna was often the only remaining structure differentiating these two zones. Figure 8 showed the extent to which this change progressed in the parenchymal vessels of case 2. It is interesting to note that in case 2 this lesion was confined to the frontal lobes, as were all the hemorrhagic phenomena. The nature of this hyaline-fibrinoid degeneration remains obscure. Spang[22] noted that the ground substance in the histiocytes described by him gave the same positive Weigert fibrin reaction as the hyaline material of Askanazy. However, as there are no subintimal infiltrations of any kind in the areas where the fibrinoid degeneration is most prominent, at least in our material, this similarity in staining properties would

seem to be merely coincidental. Isibasi[23] agreed with Abrikosoff, whom he quoted, in believing this change to be allergic in nature and somewhat similar to periarteritis nodosa. However, Isibasi admitted that he has seen fibrinoid degeneration in nontuberculous meningitis. The resemblance of periarteritis nodosa to the fibrinoid degeneration seen in our material is rather faint. Whatever the cause of this lesion, it is interesting to note that it may be sharply localized in character, and that within such an area nearly every vessel may be affected. The intracerebral vessels were far more frequently affected than the meningeal vessels, and vessels of arteriolar or precapillary caliber were the site of predilection. Fibrinoid degeneration was observed in areas where the inflammatory exudate was so acute as to consist only of polynuclear leukocytes.

SUMMARY AND CONCLUSIONS

As illustrated by the two cases of hemorrhagic tuberculous meningoencephalitis forming the basis of this study, the vascular fibrinoid degeneration of Askanazy[16] is present in the subintimal zone and media of the involved vessels. The invasion of the blood vessels begins in the periadventitial region where the cellular process is, at first, chiefly polynuclear. The media is the most resistant portion of the vessel wall. The internal elastic lamina may become fibrillar and frayed, and may separate two zones of fibrinoid material. Fibrinoid degeneration is seen more frequently in intracerebral than in meningeal vessels.

REFERENCES

1. Winkelman, N. W., and Moore, M. T. Meningeal blood vessels in tuberculous meningitis. *Am. Rev. Tuberc.*, 1940, 42, 315–333.
2. Aschoff, L. Pathologische Anatomie. G. Fischer, Jena, 1913, ed. 3, pp. 402–403.
3. Letulle, M. Anatomie Pathologique. Mason et Cie., Paris, 1931, pp. 2188 and 2198.
4. Albutt, C., and Rolleston, H. D. (eds.). A System of Medicine. Macmillan & Co., London, 1910, 8, 208.
5. Jakob, A. Normale und pathologische Anatomie und Histologie des Grosshirns. In: Aschaffenburg, G. (ed.). Handbuch der Psychiatrie. F. Deuticke, Berlin, 1929, 2, Pt. 1, 495–520.
6. Wilson, S. A. K. Neurology. (Edited by A. N. Bruce.) E. Arnold & Co., London, 1944, 1, 586–587.
7. Symmers, D. Personal communication.
8. Goldzieher, M. A. Personal communication.
9. Stevenson, L. D. Personal communication.
10. Hayem, G. Études sur les Diverses Formes d'Encéphalite (Anatomie et Physiologie Pathologiques). A. Delahaye, Paris, 1868, pp. 73–75.
11. Hoche, A. Zur Lehre von der Tuberkulose des Centralnervensystems. *Arch. f. Psychiat.*, 1888, 19, 200–228.
12. Gavazzeni, S. Eziologia ed anatomia-patologica delle encefaliti acute emorragiche. *Policlinico (sez med.)*, 1901, 8, 587–597.

13. Bombicci, G. Sull'encefalite emorragica con speciale riguardo alla forma tubercolare. *Riv. sper. di freniat.*, 1902, 28, 647–666. *Ibid.*, 1903, 29, 33–48.

14. Vanzetti, F. Contributo allo studio dell'encefalite acuta emorragica. *Arch. per le sc. med.*, 1904, 28, 385–397.

15. Grafe, E., and Gross, W. Über einen ungewöhnlichen Fall von Conglomerattuberkulose des Gehirns. *Ztschr. f. d. ges. Neurol. u. Psychiat.*, 1920, 57, 259–279.

16. Askanazy, M. Die Gefässveränderungen bei der akuten tuberkulösen Meningitis und ihre Beziehungen zu den Gehirnläsionen. *Deutsches Arch. f. klin. Med.*, 1910, 99, 333–353.

17. Kirschbaum, W. Über die Tuberkulose des Zentralnervensystems. *Ztschr. f. d. ges. Neurol. u. Psychiat.*, 1921, 66, 283–326.

18. MacGregor, A. R., Kirkpatrick, H. J. R., and Craig, W. S. Meningeal tuberculosis: bacteriology and pathology. *Edinburgh M. J.*, 1935, 42, 138–145.

19. Biber, W. Über Hämorrhagien und Gefässveränderungen bei tuberkulöser Meningitis. *Frankfurt. Ztschr. f. Path.*, 1910–11, 6, 262–311.

20. Rich, A. R., and McCordock, H. A. The pathogenesis of tuberculous meningitis. *Bull. Johns Hopkins Hosp.*, 1933, 52, 5–37.

21. Hektoen, L. The vascular changes of tuberculous meningitis, especially the tuberculous endarteritis. *J. Exper. Med.*, 1896, 1, 112–163.

22. Spang, K. Über die Gefässveränderungen bei der tuberkulösen Meningitis und ihre Bedeutung für die Rolle der subendothelialen Schicht als mesenchymales Keimlager. *Virchows Arch. f. path. Anat.*, 1936, 297, 264–270.

23. Isibasi, T. Über die fibrinoiden Gefässveränderungen bei der tuberkulösen Meningitis. *Tr. Soc. path. jap.*, 1936, 26, 68–74.

[*Illustrations follow*]

DESCRIPTION OF PLATES

Figures 1 to 7 are from case 1; Figure 8 is from case 2.

PLATE 24

FIG. 1. Right hemisphere. Of note are the clouding of the meninges by the thick exudate most marked at the base, the generalized congestion, and the confluent hemorrhages in the upper portion of the cerebellum.

FIG. 2. Large meningeal artery showing hyalinization and destruction of the vessel wall at one point (arrow), and fanwise spreading of the subintimal infiltrate from this area outward. Hematoxylin and eosin stain. $\times$ 75.

FIG. 3. Low-power view of a large artery showing the degenerative changes of the elastica interna with early aneurysmal formation in one area (arrow). Elastica and van Gieson's stains. $\times$ 85.

FIG. 4. High-power view of the same field as shown in Figure 3, showing destruction of the vessel wall, the subintimal granulation tissue, and the aneurysm. Hematoxylin and eosin stain. $\times$ 185.

Plate 24

STUDIES ON PERIARTERITIS NODOSA

II. The Rôle of Various Factors in the Etiology of Periarteritis Nodosa in Experimental Animals *

Carl C. Smith, Ph.D.,† and Pearl M. Zeek, M.D.

(From the Cardiac Laboratory of the Department of Internal Medicine and the Department of Pathology, Cincinnati General Hospital, Cincinnati, Ohio)

In a previous report [1] we described the frequent occurrence of periarteritis nodosa in rats and dogs in which hypertension had been induced by the silk-perinephritis method. [2] These findings were in agreement with the results of several other investigators who likewise had observed that in hypertensive animals, periarterial lesions were often associated with sharply rising blood pressure curves. [3-5] It was pointed out by us [1] that periarteritis nodosa had not been found in any of the 38 control rats of the same age and that no report was found in the literature of the occurrence of typical lesions in any animals in which blood pressures and kidneys had been proved to be normal. The criteria used by us in diagnosing lesions of periarteritis nodosa were detailed in our previous report. In most of the animals the lesions were widely distributed, occurring in all organs except the lungs.

Recently Rich, [6,7] in reporting 7 cases of periarteritis nodosa in man, restated the theory first suggested by Gruber [8] that this condition may occur in human beings as the result of a generalized hypersensitive reaction to some foreign agent such as serum or sulfonamides. Rich and Gregory [9] found necrotizing vascular lesions in rabbits which had been sensitized to sterile horse serum, but were unable to produce similar lesions with sulfonamides.

Since the occurrence of periarteritis nodosa in our animals was strictly limited to those which had been subjected to the silk-perinephritis operation, and since lesions were found in some rats within 1 week after operation, experiments were made to determine what factor, or factors, are associated with the operation which are capable of initiating lesions of periarteritis nodosa.

In our previous experiments [1] silk-perinephritis was produced without recourse to strict aseptic technic. Most of the reports concerning the use of this procedure in rats have not described the use of sterile precautions. [2,10,11] The region where the silk is applied quite often (but not always) became infected. With the assistance of Dr. Irvin Gibby of the Bacteriology Department, bacteriologic studies of the pus which

formed around the silk were made. Sometimes one organism was found, but often several organisms were present, most commonly staphylococci, streptococci, and *Salmonella enteritidis*. Cultures of these three types of organisms isolated from rats having typical lesions of periarteritis nodosa were used in some of the experiments to be described.

Since periarteritis nodosa was never found in any of the 38 control rats, it can be reasoned that the lesions found in these hypertensive rats developed as the result of one, or a combination, of the following factors: (a) hypersensitivity to a foreign body (silk); (b) hypersensitivity to one or more of the infectious agents often present around the silk; (c) arterial hypertension following renal manipulation; (d) release of some toxic substance from the kidney as a result of renal damage; (e) other unknown factors associated with the operation. Although several of the above possibilities cannot be conclusively ruled out by any experimental methods known at present, the results of the following experiments suggest that some of these factors have played no rôle in the production of periarteritis nodosa in these hypertensive rats.

In addition, an attempt was made to confirm the observations of Rich and Gregory[9] on rabbits made hypersensitive to horse serum. The plan, methods, results, and conclusions of the experiment on rabbits will be presented after detailing the experiments conducted on rats.

RATS

Methods

The rats used were albinos of both sexes, of the Wistar and Sprague-Dawley strains. The animals were about 6 months of age when operated upon and were fed only Purina Dog Chow Checkers and water *ad libitum*. All animals were weighed twice weekly and careful clinical notes were kept. In order to avoid the effect of frequent heating or anesthesia, required by indirect methods for measuring the blood pressure of a rat,[12,13] the only blood pressures measured in the present series were terminal ones, obtained by direct cannulation of the abdominal aorta[14] when the animal was sacrificed. Since the pressure at that time was found to vary with a number of conditions difficult to control, only limited significance has been attached to pressures thus measured.

The experiments on rats can be divided into three general types: (a) Experiments 1 and 2 consisted of introducing silk or infectious agents, or both, into the peritoneal cavity without manipulation of the kidney and without producing perinephritis; (b) In experiments 3, 4, and 5, silk alone, or silk plus some infectious material, was placed

around the kidney, producing perinephritis; (c) In experiment 6, silk plus infectious material was placed on the spleen, resulting in perisplenitis.

Experiment 1. Using sterile technic, 1 to 2 cm. bags of autoclaved silk or cellophane were placed in the abdominal cavities of 10 rats, care being taken to avoid the kidneys. These rats were sacrificed 12 weeks after operation.

Experiment 2a. A sublethal dose of a mixed suspension of three organisms, *Staphylococcus aureus*, *Streptococcus anhemolyticus* (gamma), and *Salmonella enteritidis* (all isolated from perinephric

TABLE I

Incidence of Periarteritis Nodosa in Rats Following Introduction of Silk or Infectious Agents, or Both, Distant to the Kidney

Experiment number	Experimental procedure	Periarteritis nodosa		
		Present	Absent	Questionable
1	Sterile silk or cellophane bags placed in abdominal cavity	o	10	o
2a	Intraperitoneal injection of mixed suspension of staphylococci, streptococci, and salmonellae	o	12	o
2b	Mixed suspension of the above 3 organisms in silk or cellophane bags placed in abdominal cavity	o	9	o
2c	Intraperitoneal injection of blood samples from rats showing typical lesions of periarteritis nodosa at autopsy	o	4	o
Total		o	35	o

abscesses of rats exhibiting typical periarteritis nodosa) was injected intraperitoneally into 12 normal young rats. These animals were sacrificed 32 weeks after injection.

Experiment 2b. Silk or cellophane bags containing small amounts of a suspension of the same three organisms were placed in the abdominal cavities of 9 rats, care being taken to avoid the kidneys. These rats lived from 6 days to 12 weeks following operation.

Experiment 2c. Large blood samples (4 to 5 cc.) from each of 4 rats exhibiting gross nodular lesions at autopsy were injected intraperitoneally into 4 normal young rats. These rats were sacrificed from 4 to 12 weeks following injection.

As indicated in Table I, periarteritis nodosa was not found in any of the 35 rats included in experiments 1 and 2.

Experiment 3. Using sterile technic, autoclaved silk was wrapped around one kidney of each of 26 rats; the other kidney was removed. After periods varying from 11 days to 48 weeks, these animals died or were sacrificed. Eleven rats had developed gross or microscopic evi-

dence of infection in the region where the silk had been placed. The incidence of lesions of periarteritis nodosa in the infected and non-infected rats is shown in Table II.

Experiment 4. One kidney of each of 75 rats was wrapped with silk and on the silk was placed a drop of a pure culture, diluted with broth or liquid agar, of one of the same three organisms used in experiment 2. The other kidney was either removed or in some cases left un-

TABLE II

Effect of Various Infectious Agents on the Incidence of Periarteritis Nodosa

Experiment number	Material used to produce perinephritis	Silk on one kidney and silk on other kidney, or other kidney removed			Silk on one kidney, other kidney left untouched			Total
		Periarteritis nodosa			Periarteritis nodosa			
		Present	Absent	*Questionable	Present	Absent	*Questionable	
3	Sterile silk	4	9	1	0	1	0	15
	Sterile silk which became infected	3	7	0	0	1	0	11
4	Silk infected with streptococci	0	4	1	0	8	1	14
	Silk infected with staphylococci	7	3	2	0	7	0	19
	Silk infected with salmonellae	7	3	2	0	0	0	12
	Silk infected with mixed organisms	2	2	0	0	0	0	4
5	Silk rubbed on the floor	12	4	2	0	0	0	18
Data from authors' previous report[1]	Clean silk applied without any sterile precautions	27	34	9	0	2	1	73
Total		62	66	17	0	19	2	166

*Some arteries not normal, but no full-blown necrotizing exudative lesions. Some of these cases probably are of very early or very late lesions.

touched. One-third of these rats died of peritonitis within a few days following these procedures, but 49 survived from 10 days to 45 weeks. Suspension of the organisms in liquid agar was found to give lower initial mortality. In 15 animals a mixture of all three organisms was placed on the silk; of these only 4 rats survived the acute stage and were studied.

Experiment 5. Silk contaminated by rubbing it on the laboratory floor was placed around one kidney of each of 18 rats, the other kidney being removed. This method was found to result in a high incidence of periarteritis nodosa and the initial mortality was low. These animals lived from 3 days to 40 weeks.

The results of experiments 3, 4, and 5 are shown in Table II. For

purposes of comparison, the data from our first study [1] are also included in Table II.

Experiment 6. Silk contaminated by rubbing it on the laboratory floor was wrapped around the spleen in 18 rats, producing a perisplenitis comparable to the perinephritis which resulted when similarly contaminated silk was placed around the kidney. These animals lived from 7 to 30 weeks. At autopsy the spleens were imbedded in thick collagenous hulls which contained infected pus pockets similar to those found in perinephritis. None of these 18 rats presented lesions of periarteritis nodosa either grossly or microscopically.

TABLE III

Incidence of Periarteritis Nodosa in Rats Following Various Operative Procedures

Type of operative procedure	Periarteritis nodosa			Total
	Present	Absent	Questionable	
Silk or infectious material, or both, distant to the kidney	0	35	0	35
Silk-perinephritis	62	85	19	166
Silk-perisplenitis	0	18	0	18
Controls; no operation	0	43	0	43
Total	62	181	19	262

A group of 43 rats were not operated upon but served as controls in regard to sex, age, strain, species, diet, and environment. A summary of the incidence of periarteritis nodosa following the various types of operative procedures and in the controls is shown in Table III.

Results in Rats

In experiments 1 and 2 in which silk or infectious agents, or both, were introduced into the abdominal cavities of 35 rats not adjacent to the kidneys, no periarterial lesions were noted.

When sterile silk was wrapped around the kidney and it remained sterile, or when it was infected with streptococci, there were positive lesions found in only 4 of the 29 animals comprising these groups. Three of the 4 (all originally sterile silk) were animals which survived for long periods after operation. At autopsy there was no pus, but extensive adhesions were found, which may be taken as evidence that infection may have been present at some previous time.

Of the 21 animals which had silk placed around one kidney and the other kidney left untouched, not one showed positive lesions of periarteritis nodosa grossly or microscopically. This is quite significant since in 7 of these the silk had been infected with staphylococci, while 7 of the 12 rats in which silk infected with staphylococci was placed

around one kidney and the other kidney removed had periarteritis nodosa.

Infecting the silk around one kidney with staphylococci or salmonellae or by rubbing it on the floor, and then removing the other kidney, produced lesions of periarteritis nodosa in 60 per cent of the surviving rats. On the other hand, when silk, contaminated by rubbing it on the floor, was wrapped around the spleen, a marked perisplenitis developed but no gross or microscopic evidence of periarteritis nodosa was found in any of the 18 rats studied.

These results in rats can be summarized as follows: Periarteritis nodosa occurred in a majority of the animals in which the silk around the kidney was infected with staphylococci, salmonellae, or by rubbing on the floor. No such lesions were found in rats in which the spleen was wrapped in silk similarly infected, or in which one kidney was left intact even though the other kidney was similarly infected, or in rats in which silk or similar infectious agents, or both, were introduced into the peritoneal cavity distant to the kidneys.

RABBITS

Experiment 7. Eight young albino male rabbits were divided into two groups. Those of the first group, 5 animals, were injected with 10 ml. of normal horse serum (sterile) per kg. of body weight; the second group of 3 rabbits served as untreated controls. Blood pressures of all animals were determined in triplicate on both ears by means of a Grant-Rothschild capsule.[15] Determinations were made every second day, beginning a week before the injections were made and continuing until the animals were sacrificed. Once before and three times during the period following injection of horse serum the animals were bled from the heart by cardiac puncture. Albumin-globulin ratios were measured and approximate determinations of the globulin fractions were made by means of a fractional precipitation-turbidimetric procedure.[16] Five to 6 days after the initial injection of horse serum several of the treated rabbits had flushed ears, a phenomenon previously described by Fleisher and Jones.[17] On the 28th day all rabbits were skin-tested with the original horse serum and all treated rabbits gave positive skin tests varying in degree from moderate to marked. The untreated rabbits all gave negative skin tests. On the following day all animals were given an intravenous injection of 3 ml. of the same lot of sterile horse serum. All survived and were sacrificed 2 hours after injection. Autopsies were performed and tissues from all animals were prepared for microscopic examination.

Results in Rabbits

All 5 rabbits which were injected with horse serum developed hypersensitivity, indicated by the positive skin tests and the presence of abnormal amounts of pseudoglobulin in their plasmas. Nevertheless, none of the 8 rabbits had lesions remotely suggestive of periarteritis nodosa and no evidence of glomerulonephritis was found in the kidneys of either the treated or control groups. The blood pressures, which were determined repeatedly, never varied more than 10 mm. of Hg from the original pre-treatment levels. Thus in this small group of rabbits we were unable to elicit lesions of periarteritis nodosa by producing hypersensitivity to sterile horse serum.

DISCUSSION

The occurrence of periarteritis nodosa in animals has been attributed chiefly either to the production of a sharply rising hypertension [1,3-5] or to the development of hypersensitive reactions to known or unknown antigens.[9,18,19] The following observations lend support to the first explanation:

1. Several authors have reported the frequent occurrence of periarteritis nodosa in animals in which marked kidney lesions or hypertension, or both, have been induced. Wilson and Byrom [5] produced hypertension by placing a silver clip on one renal artery; Friedman, Jarman, and Klemperer,[3] by wrapping one or both kidneys with cotton; we,[1] by wrapping one or both kidneys with silk; Ham,[20] by feeding massive doses of vitamin D which led to renal calcification; and Selye and Pentz,[4] by injecting desoxycorticosterone into unilaterally nephrectomized rats. Despite the diversity of the methods utilized in producing an elevated blood pressure, all of these investigators found that from 40 to 75 per cent of their animals developed typical lesions of periarteritis nodosa. It would appear to be more than coincidence that 95 per cent of all cases of this condition which have been reported in young rats occurred following the experimental production of hypertension by one of the several methods listed above.

2. We found in our previous study [1] that animals presenting lesions of periarteritis nodosa at autopsy had a significantly higher mean blood pressure level than did a similar group of hypertensive rats in which these lesions were not found. This difference was apparent within 1 month after operation. Similar differences in the blood pressure curves between animals with periarteritis nodosa and those without were noted by Wilson and Byrom,[5] Friedman, Jarman, and Klemperer,[3] and Selye and Pentz.[4]

Reasoning from the above theory that hypertension plays a rôle in the pathogenesis of periarteritis nodosa, one might logically ask—why, then, is periarteritis nodosa not found in every animal in which hypertension is experimentally produced? The most plausible answer would seem to be that periarteritis nodosa is not the result of hypertension alone, but rather is a very frequent complication in experimental hypertension whenever the method used in inducing the hypertension results in a sharply rising blood pressure curve, with peak pressures averaging between 200 and 240 mm. of Hg or even higher. From the limited pathologic data available it would appear that methods of inducing hypertension in rats which result in mean blood pressure levels of less than 200 mm. of Hg do not as a rule give rise to a significant incidence of periarteritis nodosa.[21,22]

The following facts are in favor of the theory that periarteritis nodosa is the result of hypersensitivity to known or unknown antigens: (1) The experimental production of hypertension usually has necessitated the introduction of a foreign body or infection, or both; (2) Rich and Gregory [9] found necrotizing vascular lesions in rabbits made hypersensitive to sterile horse serum.

Against the theory that periarteritis nodosa occurs in animals solely as a result of hypersensitivity can be listed the following facts:

1. Although the incidence of periarteritis nodosa is high in hypertensive rats, nevertheless it has been stated by Rich and Gregory,[9] and is generally accepted, that: "The well-known high resistance of the rat to anaphylactic sensitization would render this animal a particularly unsuitable subject for the experiment. Most investigators have been unable to produce either anaphylactic shock or local anaphylactic tissue damage (Arthus phenomenon) in the rat."

2. If periarteritis nodosa is the result of hypersensitivity either to the silk, cellophane, or cotton, or to the infectious agents introduced either purposefully or otherwise along with the silk, then the introduction of these materials at some point other than around the kidney should give rise to these periarterial lesions. We consider it highly significant that when silk or infectious material, or both, were placed by us in the abdominal cavity not adjacent to the kidney, or were placed adjacent to the testicles by Friedman, Jarman, and Klemperer,[3] no lesions were found. When silk which had been rubbed upon the laboratory floor was placed around the spleens of 18 rats, producing a marked chronic suppurative perisplenitis, no lesions of periarteritis nodosa occurred. Yet when silk similarly rubbed on the floor was placed on one kidney of each of 18 rats and the opposite kidney removed, 12 animals developed typical lesions of periarteritis nodosa.

3. Although data concerning the distribution of lesions of periarteri-

tis nodosa in various organs of rats and human beings will be presented in detail in a subsequent report, the fact that typical periarteritis nodosa was not found in the pulmonary arterial tree appears highly significant. Its complete absence in the lungs is difficult to account for on the basis of differential hypersensitivity, and can much more easily be explained by the fact that the blood pressure in the pulmonary circuit is relatively low and is not significantly raised in experimental hypertension.[23]

4. In a small but carefully controlled experiment we were unable to produce typical periarteritis nodosa in rabbits by sensitizing them to sterile horse serum. In a much larger series of rabbits made hypersensitive to horse serum, Fox and Jones [24] also were unable to confirm the observations of Rich and Gregory.[9] Most of the animals presenting periarteritis nodosa, reported by Rich and Gregory, were also found to have glomerulonephritis. This lesion is commonly associated with hypertension, but no blood pressure determinations were made on their rabbits. Therefore, evaluation of the rôle of hypersensitivity in the pathogenesis of experimental periarteritis nodosa should be reserved until it has been demonstrated that periarteritis nodosa can be produced by hypersensitizing animals in which the blood pressure remains within normal limits and in which the kidneys present no complicating lesions.

SUMMARY

Experiments have been performed to determine what factor, or factors, associated with the silk-perinephritis method of producing hypertension in rats, are capable of initiating lesions of periarteritis nodosa.

Typical lesions occurred in a majority of unilaterally nephrectomized animals in which the silk around the remaining kidney was infected with staphylococci or salmonellae, or by rubbing the silk on the floor. No such lesions were found in rats in which the spleen was wrapped in silk similarly infected, or in which one kidney was left intact even though the other kidney was similarly infected, or in rats in which silk or similar infectious agents, or both, were introduced into the peritoneal cavity distant from the kidney.

No lesions of periarteritis nodosa were found in 5 rabbits which had been made hypersensitive to sterile horse serum.

The available data support the hypothesis that periarteritis nodosa is associated with sharply rising hypertension, and tend to refute the theory that this condition is the result of hypersensitivity to known or unknown antigens.

REFERENCES

1. Smith, C. C., Zeek, P. M., and McGuire, J. Periarteritis nodosa in experimental hypertensive rats and dogs. *Am. J. Path.*, 1944, 20, 721–735.

2. Kempf, G. F., and Page, I. H. Production of experimental hypertension and the indirect determination of systolic arterial pressure in rats. *J. Lab. & Clin. Med.*, 1942, 27, 1192–1196.

3. Friedman, B., Jarman, J., and Klemperer, P. Sustained hypertension following experimental unilateral renal injuries. Effects of nephrectomy. *Am. J. M. Sc.*, 1941, 202, 20–29.

4. Selye, H., and Pentz, E. I. Pathogenetical correlations between periarteritis nodosa, renal hypertension and rheumatic lesions. *Canad. M. A. J.*, 1943, 49, 264–272.

5. Wilson, C., and Byrom, F. B. Vicious circle in chronic Bright's disease. Experimental evidence from hypertensive rat. *Quart. J. Med.*, 1941, 10, 65–93.

6. Rich, A. R. The rôle of hypersensitivity in periarteritis nodosa, as indicated by seven cases developing during serum sickness and sulfonamide therapy. *Bull. Johns Hopkins Hosp.*, 1942, 71, 123–140.

7. Rich, A. R. Additional evidence of the rôle of hypersensitivity in the etiology of periarteritis nodosa. Another case associated with a sulfonamide reaction. *Bull. Johns Hopkins Hosp.*, 1942, 71, 375–379.

8. Gruber, G. B. Zur Frage der Periarteriitis nodosa, mit besonderer Berücksichtigung der Gallenblasen- und Nieren-Beteiligung. *Virchows Arch. f. path. Anat.*, 1925, 258, 441–501.

9. Rich, A. R., and Gregory, J. E. The experimental demonstration that periarteritis nodosa is a manifestation of hypersensitivity. *Bull. Johns Hopkins Hosp.*, 1943, 72, 65–88.

10. Cromartie, W. J. Arteritis in rats with experimental renal hypertension. *Am. J. M. Sc.*, 1943, 206, 66–75.

11. Grollman, A., and Williams, J. R., Jr. Experimental chronic hypertension in the rat. *Am. J. M. Sc.*, 1942, 204, 73–79.

12. Byrom, F. B., and Wilson, C. Plethysmographic method for measuring systolic blood pressure in the intact rat. *J. Physiol.*, 1938, 93, 301–304.

13. Williams, J. R., Jr., Harrison, T. R., and Grollman, A. A simple method for determining the systolic blood pressure of the unanesthetized rat. *J. Clin. Investigation*, 1939, 18, 373–376.

14. Grossman, E. B., and Williams, J. R., Jr. Relation of age to renal pressor substance. *Arch. Int. Med.*, 1938, 62, 799–804.

15. Grant, R. T., and Rothschild, P. A device for estimating blood-pressure in the rabbit. *J. Physiol.*, 1934, 81, 265–269.

16. Smith, C. C. Unpublished observations.

17. Fleisher, M. S., and Jones, L. Serum sickness in rabbits. I Manifestations of serum sickness. *J. Exper. Med.*, 1931, 54, 597–613.

18. Masugi, M. Zur Pathogenese der diffusen Glomerulonephritis als allergischer Erkrankung der Niere. *Klin. Wchnschr.*, 1935, 14, 373–376.

19. Metz, W. Die geweblichen Reaktionserscheinungen an der Gefässwand bei hyperergischen Zuständen und deren Beziehungen zur Periarteriitis nodosa. *Beitr. z. path. Anat. u. z. allg. Path.*, 1931, 88, 17–36.

20. Ham, A. W. Coronary and aortic sclerosis, periarteriitis nodosa, chronic nephritis and hypertension as sequelae to a single experimentally produced widespread calcium precipitation in the rat. *Arch. Path.*, 1940, 29, 731.

21. Chanutin, A., and Ferris, E. B., Jr. Experimental renal insufficiency produced by partial nephrectomy. I. Control diet. *Arch. Int. Med.,* 1932, 49, 767–787.

22. Grollman, A., Harrison, T. R., and Williams, J. R., Jr. The mechanism of experimental renal hypertension in the rat: the relative significance of pressor and anti-pressor factors. *Am. J. Physiol.,* 1943, 139, 293–298.

23. Katz, L. N., and Steinitz, F. S. Pulmonary arterial pressure in experimental renal hypertension. *Am. J. Physiol.,* 1939–40, 128, 433–439.

24. Fox, R. A., and Jones, L. R. Vascular pathology in rabbits following administration of foreign protein. *Proc. Soc. Exper. Biol. & Med.,* 1944, 55, 294–295.

THE NONPORTAL DISTRIBUTION OF THE TRABECULAE IN DIETARY CIRRHOSIS OF RATS AND IN CARBON TETRACHLORIDE CIRRHOSIS OF RATS AND GUINEA-PIGS *

L. L. Ashburn, M.D., K. M. Endicott, M.D., F. S. Daft, Ph.D., and R. D. Lillie, M.D.

(From the Pathology Laboratory and the Division of Physiology, National Institute of Health, U. S. Public Health Service, Bethesda, Md.)

The relationship of choline, cystine, and methionine to necrosis and cirrhosis of the liver in rats has been investigated in a number of laboratories.[1-7] These two conditions now have been clearly identified and established as separate entities.[2,6] However, there still exist differences of opinion as to the architectural relationship of the connective tissue trabeculae in the cirrhotic livers produced by dietary deficiency of choline. Most workers have described the fibrous tissue as being formed in, or emanating from, the portal areas. This description corresponds to that of portal cirrhosis in man. In a previous report describing the histogenesis of dietary cirrhosis in rats,[8] we stated that the trabeculae of ceroid and connective tissue formed along the hepatic venules and only later abutted on portal areas. This interpretation does not permit the classification of the process as portal cirrhosis.

In order to test this interpretation, it was decided to continue the investigation of this subject using precise means of locating the position of the trabeculae. For this purpose, hepatic or portal veins were injected with a charcoal mass similar to that used by Mall[9] in his classic investigations on the hepatic lobule.

The centrolobular distribution of necrosis and fatty degeneration of the liver in CCl_4 intoxication is well recognized. However, the location of the subsequently formed connective tissue trabeculae is a controversial point. Therefore, the histogenesis of this process was investigated in the same manner as that of the dietary cirrhosis.

Experimental Procedure

Forty-three albino rats at weaning were started on diet no. 545 which had the following composition: Leached casein, 4 per cent; cystine, 0.5 per cent; cod-liver oil, 2 per cent; Wesson oil, 3 per cent; Osborne and Mendel salt mixture,† 4 per cent; corn starch, 86.5 per cent. A supplement of 100 μg. of thiamin chloride, 50 μg. of riboflavin, 20 μg. of pyridoxine, 50 μg. of calcium pantothenate, and 1 mg. of

nicotinic acid was given to each rat daily. All rats received 20 per cent alcohol as a source of fluid. In order to obtain livers showing variable degrees of pathologic alteration, the rats were killed after having been under experimental conditions from 50 to 150 days. The livers were injected with charcoal gelatin mass through either the hepatic or portal veins.

Nineteen adult albino rats, weighing from 248 to 388 gm., were injected subcutaneously with carbon tetrachloride twice a week. The dose of carbon tetrachloride was 0.05 cc. per 100 gm. of initial body weight. The same dose was continued for the duration of the experiment regardless of the weight changes. These rats were fed an *ad libitum* diet of stock pellets and water. The rats were killed at intervals from 21 to 87 days and the livers were injected with charcoal gelatin mass.

In another experiment, guinea-pigs fed a stock vegetable diet received repeated subcutaneous injections of carbon tetrachloride at various dosage levels. Some of these animals developed cirrhosis. The livers of 9 were injected with the mass.

Preparation of Injection Mass. The injection mass is made just before use by dissolving 8 gm. of gelatin in 100 cc. of hot water and adding 20 gm. of animal charcoal. While the animal is being prepared for injection, the mass is kept in a conical glass in a warm water bath (about 55° C.). The charcoal is kept in suspension by use of a glass agitator rod of propeller type, powered by a small electric motor having an adjustable friction reduction gear.

Preparation of Animal and Injection of Liver. The animal is killed with chloroform and attached to a small wooden autopsy board. Anterior abdominal and thoracic walls are removed, and absorbent cotton is folded over the cut ends of the ribs to prevent injury to the liver. An obstructing tie is made around the inferior vena cava at any convenient point below the liver, usually just above or below the right renal vein. When it is desired to inject the hepatic veins, a glass cannula is inserted into the thoracic inferior vena cava through a slit made in the right auricle and tied in place. The cannula is attached by a 6 inch length of small rubber tubing to a 100 cc. syringe containing warm saline solution. After cutting the portal vein, gentle pressure on the syringe plunger is exerted to wash the blood from the liver. Most of the blood is quickly removed by this method. In some livers the blood remains in a few patches. The removal of this blood is effected by gently stroking the liver surface with the moist fingertips while continuing the irrigation. After the blood has been washed out, the charcoal gelatin mass is injected through the same tubing by replacing the syringe

containing saline solution with a 10 cc. syringe containing the mass. In the transfer of syringes, care should be taken to avoid the inclusion of air bubbles. The amount of pressure necessary to inject the hepatic tree is slight and is controlled by observation of the degree of filling of the subcapsular terminal branches of the hepatic veins. When the veins are filled, additional pressure may rupture the hepatic vein at the hilus. Injection of the intrahepatic portal veins is accomplished in a similar manner, the cannula being tied in the portal vein in any accessible location. The inferior vena cava is cut in the thorax to allow escape of the blood and saline solution. Since the terminal branches of the portal vein do not reach the surface of the liver, only a grayish color results from the injection. The injection is stopped when the system will not, with gentle pressure, receive more of the mass. Livers are injected either by hepatic or by portal veins, never by both. Although some of the gelatin passes through the hepatic lobules, the carbon does not, and is limited to the portal or hepatic systems, as the case may be. It should be noted here that occasionally some venous branches will not be filled by the mass.

After the injection is completed, the cannulized vessel is clamped and the entire liver removed and placed in the fixative, which in this study was either 10 per cent formalin or Zenker's fluid. The specimen is then placed in the refrigerator to accelerate the hardening of the mass. After 1 to 2 hours, the livers may be sliced at 3 to 4 mm. intervals, and fixation continued at room temperature.

For this study all tissue was embedded in paraffin. Sections were stained for connective tissue by Mallory's aniline blue and van Gieson's picrofuchsin methods. Lillie's modification of the di-ammine silver hydroxide method * was used for demonstrating reticulum.

DISTRIBUTION OF TRABECULAE IN DIETARY CIRRHOSIS

In the dietary cirrhotic livers in which the charcoal gelatin mass was introduced through the hepatic vein, the injected veins were regularly included in, and connected by, the connective tissue trabeculae. In the livers injected through the portal veins, the injected veins were usually not in, or connected by, the trabeculae.

As previously reported,[8] the early deposition of fat was most marked about hepatic venules. Ceroid soon appeared in liver cells and in groups of phagocytes about hepatic veins and venules. These phagocytes became incorporated into loosely fibrous trabeculae. The trabeculae

---

* Lillie, R. D. A simplified method of preparation of di-ammine-silver hydroxide for reticulum impregnation; comments on the nature of so-called sensitization before impregnation. *Stain Technol.*, 1946, 21, 69–72.

formed not only about the central vein but also along the sublobular and large branches of the hepatic veins. In fact, in some instances the first increase in connective tissue to be noted appeared around these larger vessels, more particularly near the hilar portion of the lobes.

In comparing portal versus hepatic distribution of fibrous tissue, one should compare similar levels of the two systems. This tends to lessen confusion in interpretation of the histologic picture. The point where the recognition of the terminal radicles of portal and hepatic veins is easiest is in the subcapsular layer of lobules. Here, where sections are made perpendicular to the surface, alternating central veins and portal veins of the same order can often be seen. It was apparent in the present study that the trabeculae coursed along these hepatic veins and reached and merged with the capsule, as is evident in Figure 2. It is significant that hepatic veins normally reach the capsule, whereas portal veins do not, a fact reported by Mall,[9] Figure 1. In deeper lobules the trabeculae are also seen to connect injected hepatic veins. Parts of three lobules with their constituent noninjected and uninvolved portal areas are shown in Figure 6. Part of the apparent disproportionate size of the portal and hepatic veins is due to distention of the latter by the injection mass.

Some trabeculae seen in livers injected through the hepatic vein did not contain the mass. This may be explained by the failure of the mass to reach the area due to narrowing of the vein or blockage at a lower level by an aggregate of charcoal particles. To determine how trabeculae were related to portal areas, it was necessary to study cirrhotic livers in which the charcoal mass was introduced through the portal vein. Figures 7 to 10 show injected portal veins in uninvolved portal areas. These lobules are surrounded by trabeculae containing no mass. Such relationship was found with regularity in those livers in which the cirrhotic process had not progressed to the point of extreme disarrangement of lobular architecture. Even in these, isolated nodules of hepatic parenchyma often showed uninvolved portal areas with injected veins (Fig. 11). In livers showing fairly advanced cirrhosis, portal areas were found at the margins of trabeculae and sometimes incorporated within them (Fig. 13). These portal areas contained vessels of relatively large size and obviously were not related directly to the functional liver unit. It was clear in some instances that a trabecula passed by, abutted on, or enclosed a portal area because destruction of one or more lobules had brought hepatic and portal vessels close together. Also the larger hepatic veins pursued an independent course to their exit from the liver,[10] a circumstance which in some instances brought the larger hepatic and portal vessels in close relationship at certain

points. Such a condition would account for the inclusion of an occasional portal area in a trabecula which was primarily related to the hepatic vein. In the extensively involved livers, some trabeculae extended to and incorporated the larger portal areas, and when such occurred the fibroblasts of these areas undoubtedly contributed to the new growth of connective tissue. In areas where almost complete replacement of parenchyma had taken place, usually near the hilus, injected portal veins of medium and large size were seen irregularly scattered throughout the ceroid and connective tissue mass.

Proliferation of small bile ducts is a feature of this cirrhosis which varies greatly in degree in different rats. It is usually prominent only in some of the animals showing moderately severe or severe damage. The presence of these ducts in the trabeculae served to confuse the picture and make interpretation difficult. That these bile ducts were present in the trabeculae, which in this study were shown to follow and connect hepatic veins, was evident; and the explanation for their presence was also evident in some preparations. These small ducts were seen extending from the portal area through the lobule between cell cords and reaching the trabeculae (Fig. 11). In most instances many more ducts were present in the trabeculae than were seen traversing the lobules. That these represented growth with tortuosity or budding in their abnormal location seems reasonable. In those livers where cirrhosis had proceeded to complete replacement of certain lobules, the reason for the presence of bile ducts in the proliferated connective tissue was obvious. This was seen particularly toward the base of the lobes and in diffuse or patchy subcapsular areas.

DISTRIBUTION OF TRABECULAE IN CARBON TETRACHLORIDE CIRRHOSIS OF THE LIVER

In hepatic cirrhosis induced by carbon tetrachloride the fatty degeneration and necrosis in both rats and guinea-pigs occurred around the hepatic veins, mainly the terminal branches (centrolobular) (Fig. 12). The trabeculae of fibrous tissue formed in the same location. These trabeculae and their relationship to the injected hepatic veins are shown in Figures 15 to 20. Their distribution was essentially the same as that already described in more detail for dietary cirrhosis.

The demonstration that the connective tissue trabeculae in cirrhosis due to CCl_4 follow and connect hepatic veins rather than the portal areas simplifies the understanding of the pathogenesis of this condition. The connective tissue proliferates in the same areas where damage to the liver takes place. Since this is true, it is unnecessary to assume, as has been suggested, the presence of an autolysate which travels in the

lymphatics from areas of necrosis to the portal areas, and there stimulates the production of connective tissue.

DISCUSSION

The hepatic lobule is generally considered as having a terminal radicle of the hepatic vein at its center and being bounded peripherally by an imaginary line passing through the associated portal areas. This conception is based largely on the fact that in swine a connective tissue capsule is found in this location, thus enclosing a mass of liver cells—the hepatic lobule. In the seal, hepatic lobules are also well demarcated from one another, but in this animal it is a different lobule that is outlined.[11] Microscopically, it is seen that the hepatic veins are at the periphery, where, as lacunae or rectilinear venous sinuses, they form walls of separation of the portal lobule. Theile[12] demonstrated in the dog and rabbit the presence of cleavage planes between portal lobules. This was done by crushing, and washing the livers of these animals in a stream of water. When treated thus, the whole system of lobules became isolated and were clustered around the branches of the portal veins. The foregoing are cited to indicate that from a standpoint of architecture and comparative anatomy it should be possible to produce cirrhosis of the liver in which the trabeculae form at the periphery of either the hepatic or portal lobule, the type produced probably depending on the location of the injury.

The concept that the connective tissue proliferation occurs in the same location as the injury is supported by the fact that in carbon tetrachloride cirrhosis of rats and guinea-pigs, fatty degeneration and necrosis occur about the central veins and the fibrous tissue trabeculae form in and connect these areas. Further support for this concept is found in dietary cirrhosis. In the development of this condition, the deposition of fat usually occurs first, and is most pronounced, around the hepatic veins. This is followed by the accumulation of ceroid and the formation of connective tissue strands which follow and connect these hepatic veins.

SUMMARY

Albino rats at weaning were placed on a cirrhosis-producing diet and killed after 50 to 150 days. The livers were injected through the portal or hepatic veins with charcoal gelatin mass to mark these structures effectively in the microscopic preparation. Histologic study showed that the fatty deposition, ceroid accumulation, and fibrous trabeculation primarily followed and connected hepatic veins. In livers showing marked alteration, the trabeculae sometimes coursed by, or abutted on,

large portal areas. However, even in these livers the portal areas comparable in level to the centrolobular veins were not primarily related to the trabeculae.

In other experiments, cirrhosis of the liver of rats and guinea-pigs was produced by the repeated subcutaneous administration of carbon tetrachloride. The livers were injected with the charcoal gelatin mass and studied histologically. The connective tissue trabeculae occurring in these livers were primarily related to hepatic veins and showed essentially the same distribution as that seen in the dietary cirrhosis.

REFERENCES

1. Blumberg, H., and McCollum, E. V. The prevention by choline of liver cirrhosis in rats on high fat, low protein diets. *Science*, 1941, 93, 598–599.
2. Daft, F. S., Sebrell, W. H., and Lillie, R. D. Prevention by cystine or methionine of hemorrhage and necrosis of the liver in rats. *Proc. Soc. Exper. Biol. & Med.*, 1942, 50, 1–5.
3. György, P., and Goldblatt, H. Observations on the conditions of dietary hepatic injury (necrosis, cirrhosis) in rats. *J. Exper. Med.*, 1942, 75, 355–368.
4. Webster, G. T. Cirrhosis of the liver among rats receiving diets poor in protein and rich in fat. *J. Clin. Investigation*, 1942, 21, 385–392.
5. Engel, R. W. Liver cirrhosis and choline. *Federation Proc.*, 1943, 2, 62.
6. Himsworth, H. P., and Glynn, L. E. Massive hepatic necrosis and diffuse hepatic fibrosis (acute yellow atrophy and portal cirrhosis): Their production by means of diet. *Clin. Sc.*, 1944, 5, 93–123.
7. Hock, A., and Fink, H. Über eine schwere ernährungsbedingte Stoffwechselstörung und ihre Verhütung durch Cystin. *Ztschr. f. physiol. Chem.*, 1943, 278, 136.
8. Lillie, R. D., Ashburn, L. L., Sebrell, W. H., Daft, F. S., and Lowry, J. V. Histogenesis and repair of the hepatic cirrhosis in rats produced on low protein diets and preventable with choline. *Pub. Health Rep.*, 1942, 57, 502–508.
9. Mall, F. P. A study of the structural unit of the liver. *Am. J. Anat.*, 1906, 5, 227–308.
10. Maximow, A. A., and Bloom, W. A Textbook of Histology. W. B. Saunders Co., Philadelphia & London, 1930.
11. Brissaud and Sabourin. Sur la constitution lobulaire du foie et les voies de la circulation sanguine intra-hépatique. *Compt. rend. Soc. de biol.*, 1888, 40, 757–762.
12. Theile, F. W. T. (Cited by Mall.[9])

[*Illustrations follow*]

DESCRIPTION OF PLATES

PLATE 26

FIG. 1. Dietary cirrhosis, rat. Gross photograph of liver injected with charcoal gelatin mass through hepatic vein. No gross evidence of cirrhosis.

FIG. 2. Dietary cirrhosis, rat. Liver showing increase in connective tissue around the injected hepatic vein. The connective tissue follows the vein to, and blends with, the capsule at the point of retraction. Reticulum stain. $\times$ 80.

FIGS. 3, 4, and 5. Dietary cirrhosis, rat. Relationship of trabeculae to the injected hepatic veins is shown. Ceroid appears in the trabeculae as small refractile globules. Van Gieson's stain. $\times$ 50.

FIG. 6. Dietary cirrhosis, rat. Injected hepatic veins incorporated in, and connected by, trabeculae. Parts of three lobules are shown; of note are the uninjected portal veins outlined by reticulum. Reticulum stain. $\times$ 50.
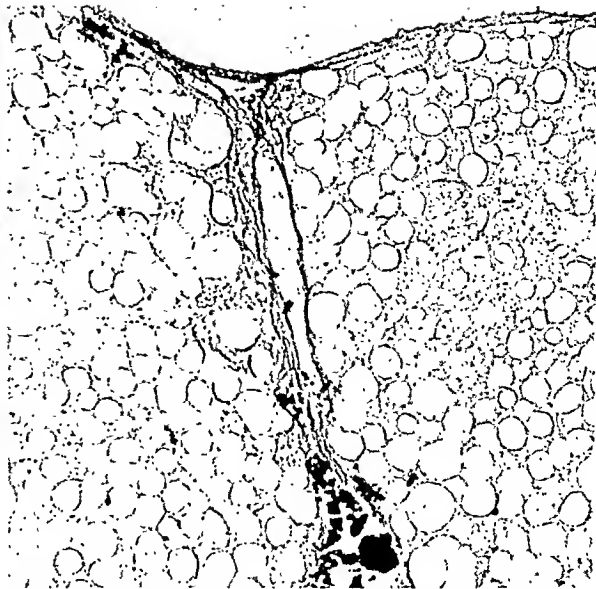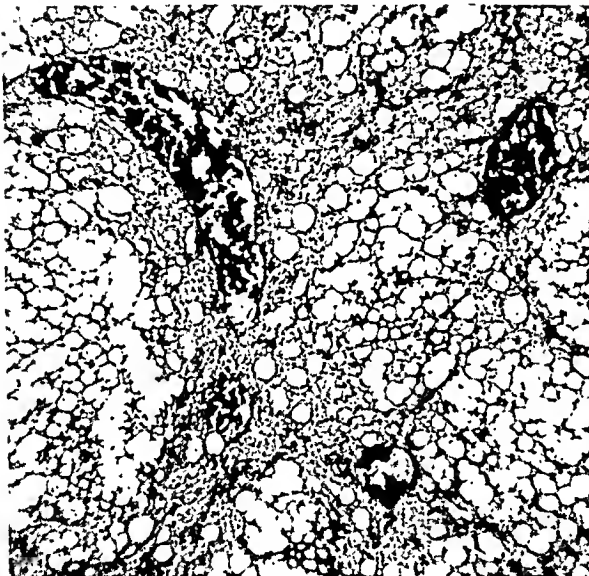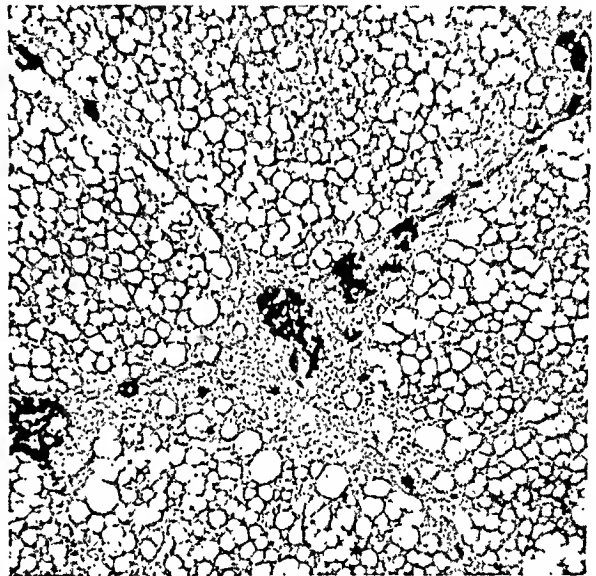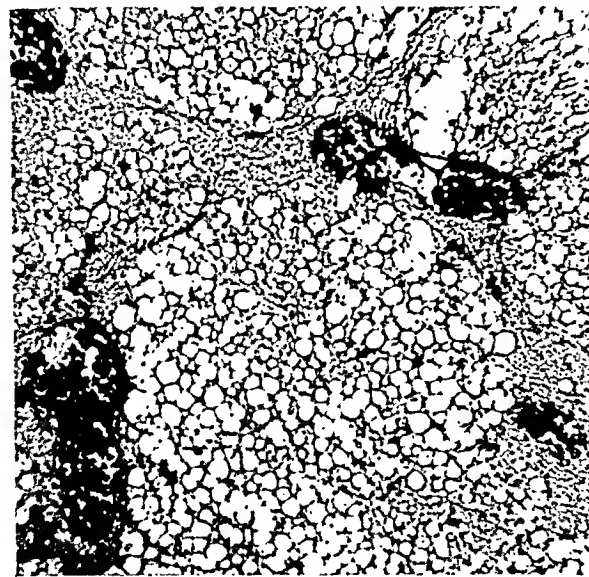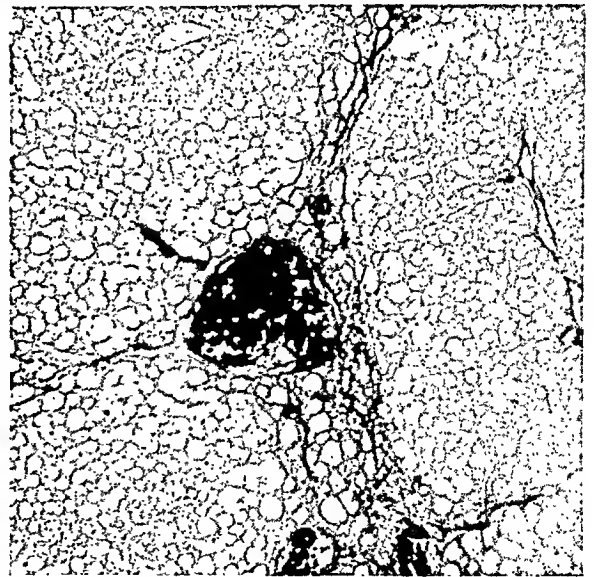
PLATE 27

FIGS. 7, 8. and 9. Dietary cirrhosis in a rat, with portal injection. Mallory's aniline blue stain. × 50.

FIG. 10. Dietary cirrhosis in a rat, with portal injection. Picrofuchsin stain. × 50.

FIG. 11. Dietary cirrhosis in a rat, with portal injection. Isolated nodule of liver. Hematoxylin and eosin stain. × 250.

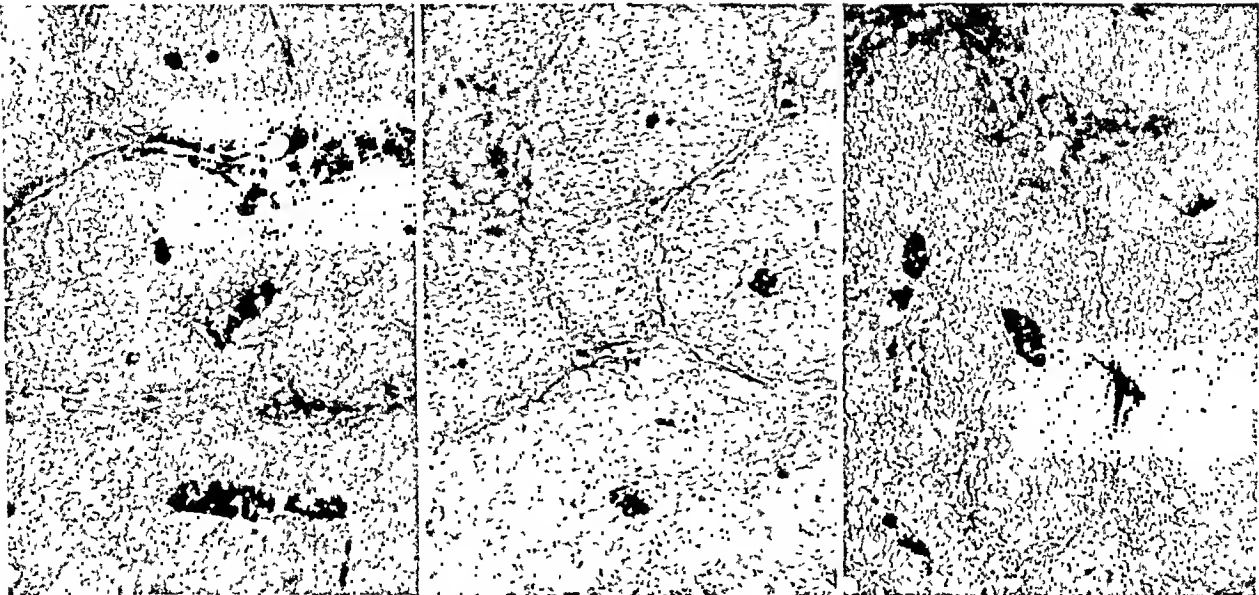FIG. 12. Carbon tetrachloride cirrhosis in a rat, with portal injection. Frozen section, stained with oil red O. × 50.

FIG. 13. Dietary cirrhosis in a rat, with portal injection. Narrow expansions from the thick trabeculae abut on the large portal area. The small portal area is unrelated to the proliferated connective tissue. Mallory's aniline blue stain. × 50.

FIG. 14. Carbon tetrachloride cirrhosis in a rat, with portal injection. Van Gieson's stain. × 50.
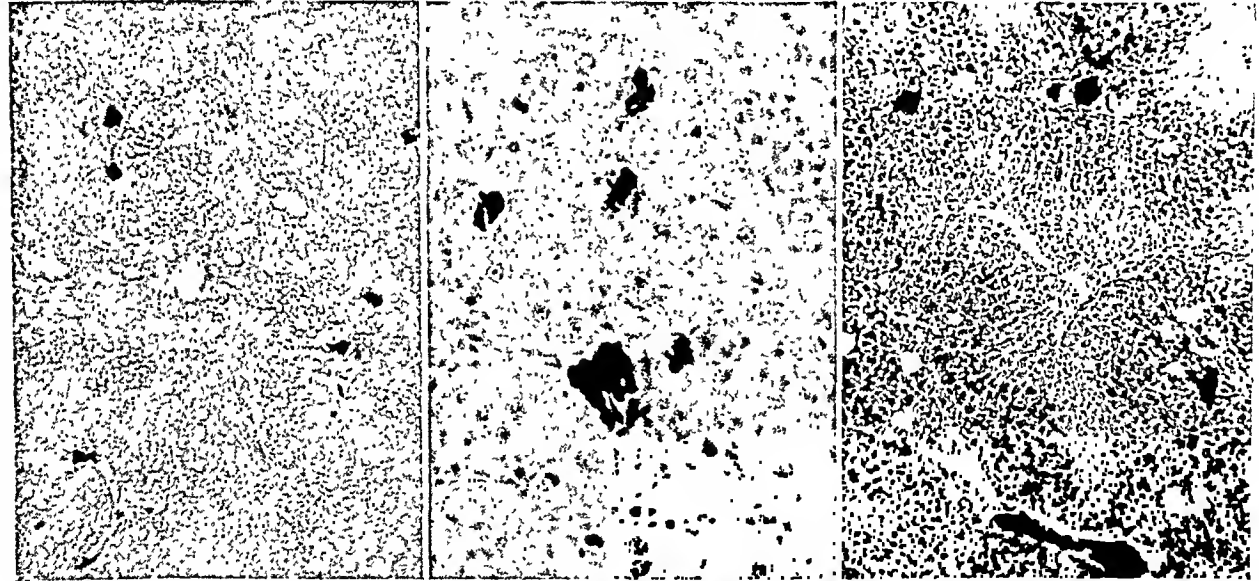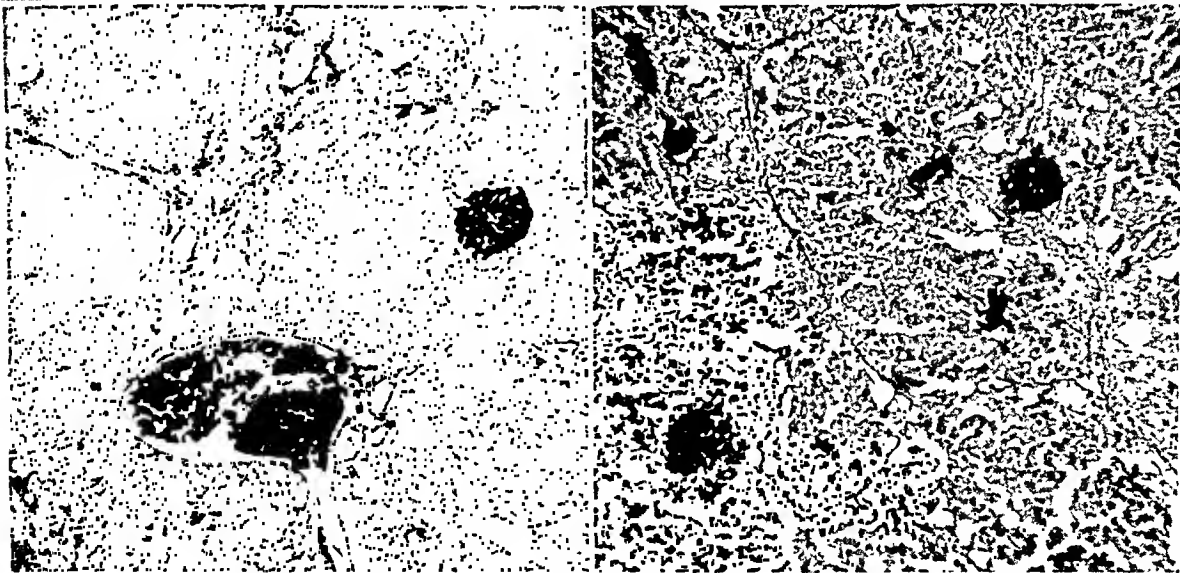
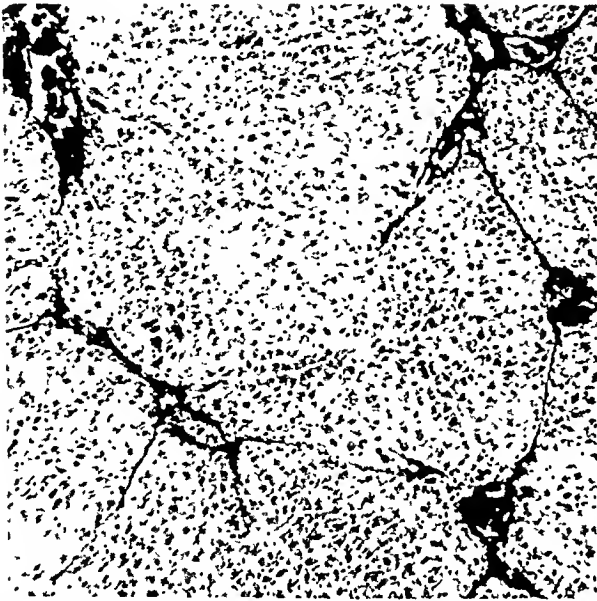Ashburn, Endicott, Daft, and Lillie

Nonportal Trabeculae in Experimental Cirrhosis
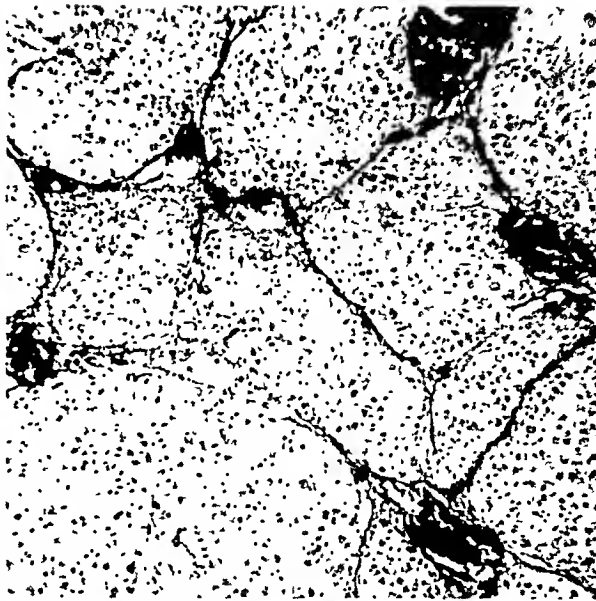
PLATE 28

FIGS. 15, 16, 17. and 18. Carbon tetrachloride cirrhosis in a rat: hepatic veins injected. There is considerable alteration of lobular architecture. Trabeculae follow and connect hepatic veins. Van Gieson's stain. × 50.

FIGS. 19 and 20. Carbon tetrachloride cirrhosis in a guinea-pig; hepatic veins injected. Injected veins are included in, and connected by, thin trabeculae. Van Gieson's stain. × 50.
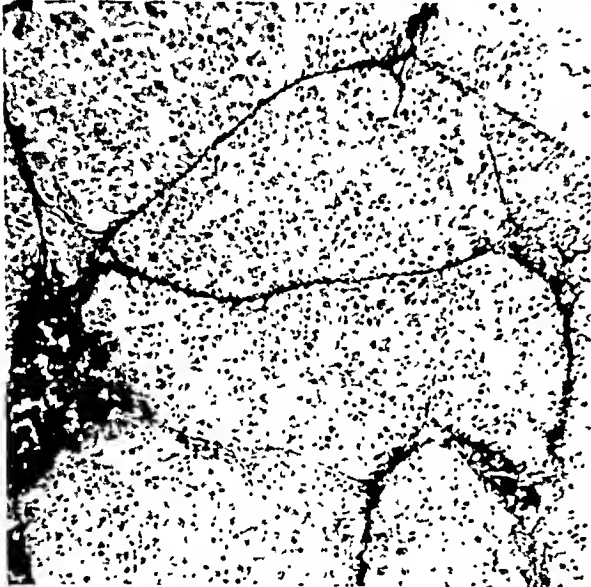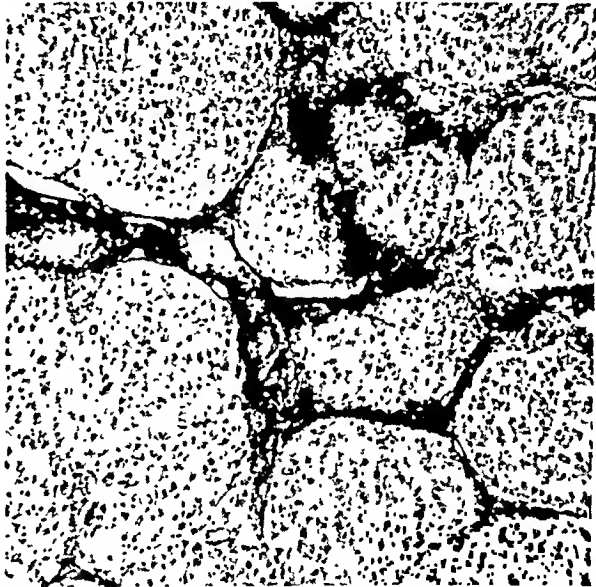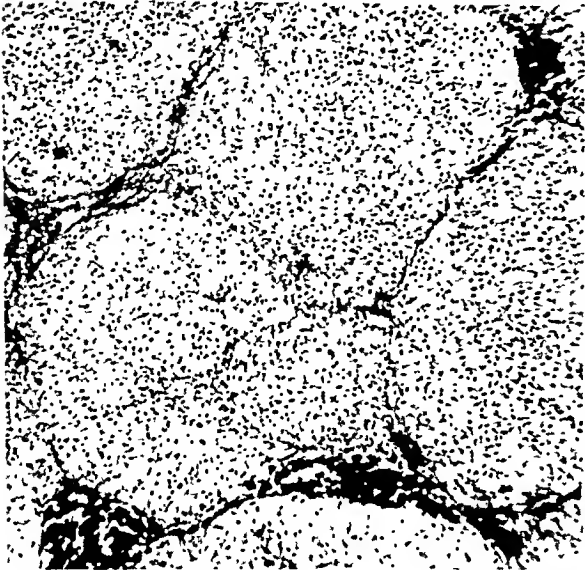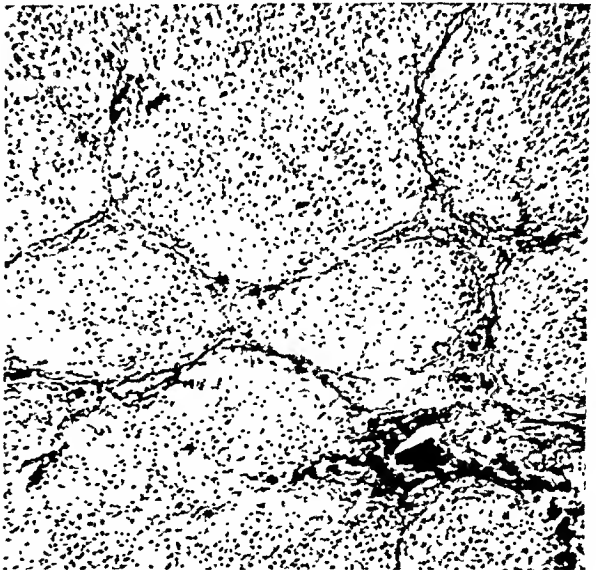
PLATE 28

15

16

17

18

19

20

Ashburn, Endicott, Daft, and Lillie

Nonportal Trabeculae in Experimental Cirrhosis

THE PATHOLOGY OF HIGH-ALTITUDE FROSTBITE *

Major NATHAN B. FRIEDMAN, M.C., A.U.S., and Major ROBERT A. KRITZLER, M.C., A.U.S.

(*From the Army Institute of Pathology, Washington 25, D.C., and the Second General Hospital*)

This study of the morbid anatomy of frostbite incurred by airmen exposed to severe cold at high altitudes is the second in a series of reports dealing with the reactions of tissues to injury by cold. It is based on material received at the Army Institute of Pathology from military sources.†

Frostbite of aviators was noted during the First World War [1,2] and the Spanish Civil War,[3] but until bombing from high altitudes had become a regular practice in World War II it was not a common condition. Among men of the Eighth Air Force high-altitude frostbite was at one time the second most frequent battle injury.[4] The experience of the Army Air Force with this problem has been recorded by Davis, Scarff, Rogers, and Dickinson [5] and that of the Royal Air Force by Cade.[6]

CLINICAL DATA

The 20 patients from whom material was obtained for this study (Table I) incurred frostbite during raids in B-17 and B-24 bombers at altitudes of 20,000 to 27,500 feet and at temperatures of −30° to −50° C. The men were in their late teens or early twenties and with one exception were white Americans. The majority were tail gunners, but waist and turret gunners are included in the group. Five had incurred frostbite during previous missions.

The briefest exposure was 1 minute and the longest 5 hours. The usual reason for exposure was removal of electrically heated gloves to deal with a frozen oxygen mask or tubing, but burned-out wiring and

---

damage to equipment by flak were sometimes responsible. Six men had anoxia at the time of exposure; the reactions to lack of oxygen ranged in severity from partial blackout to prolonged coma. The anoxia is of interest because inadequate oxygenation has been thought to augment injury by cold.[5]

The fingers, affected more often than other parts of the body, were involved in this order of frequency: fourth, index, third, second, and thumb. One man had frostbite of the toes and 4 had damage to the face in addition to injury of the fingers. Sheeley[7] reported that frostbite of the face of aviators remained a problem after the incidence of frostbite of the fingers had been reduced. One patient had frostbite of the buttock (Fig. 1), as a consequence of urinating into his flying suit.

During exposure the fingers became numb, stiff, shrunken, and hard; they were described as yellow-white and waxy. As the frostbitten fingers thawed, in the navigator's compartment or radio room, during descent of the plane or after landing, pain and swelling set in. Immersion of the fingers in cold water deferred the edematous reaction, but after the hands were removed from the water cyanotic discoloration or redness, edema, and blisters (Fig. 2) appeared. Bullae sometimes developed a few hours after exposure, especially if the hands had been excessively warmed; they were almost invariably present within 24 hours.

Damage of the mildest type resulted only in shedding of the skin and nails; there was no significant loss of tissue. Severe injury was followed by gangrene of the digits (Figs. 3 and 4), sometimes as soon as 8 days after exposure, although usually 2 to 3 weeks were required for its full development.

MICROSCOPIC CHANGES

Most of the material consisted of amputated gangrenous fingers, which showed only late lesions. In a few instances tissue was taken for biopsy prior to amputation.

Early Lesions

The description of the early lesions is based primarily on the study of a specimen obtained for biopsy from a finger 3 days after exposure. The finger subsequently became gangrenous.

The specimen included the roof of a vesicle and a portion of the subcutaneous tissue. The space formed by cleavage at the dermo-epidermal junction contained precipitated protein, leukocytes, and red cells. The outer epithelial layers were necrotic, while in many places the inner portion of the malpighian layer and the basal layer were

TABLE I

Data from Twenty Cases of High-Altitude Frostbite

Case number	Age of patient	Patient's position in plane	Altitude, thousands of feet	Temperature	Circumstances of exposure	Duration of exposure	Complications	Number of days to		Portion of body involved
								Gangrene	Amputation	
1	22	Tail gunner	26	−30°C.	Oxygen mask froze	1 hour	Anoxia	15	47	Fingers
2		Tail gunner	27	−45°C.	Ungloved hand; Flak cut wiring of heated suit	Few minutes	Anoxia; abdominal wound	12		Fingers
3							Head wound		42	Fingers
4	23	Tail gunner	26.5	−45°C.	Oxygen mask froze		Anoxia	15		Fingers
5	19	Tail gunner	24		Insufficiently heated gloves; Gloves failed to heat			27		Fingers
6	22	Tail gunner	27		Oxygen mask froze		Anoxia			Fingers
7	24	Turret gunner		−48°C.	Insufficiently heated gloves; Gloves failed to heat	5 hours	Previous frostbite		42	Fingers, toes, cheeks
8	22				Disconnected suit; Hydraulic fluid froze on gloves			8	42	Fingers, cheeks
9	23				Oxygen mask froze		Previous frostbite		56	Fingers
10					Insufficiently heated gloves; Suit burned out; No gloves	4 hours	Previous frostbite	8	55	Fingers
11					Oxygen mask froze; Insufficiently heated gloves; Gloves failed to heat				59	Fingers
12	20	Tail gunner	26.5	−45°C.	Oxygen mask froze; Removed gloves	1 hour	Previous frostbite	27	71	Fingers
13		Tail gunner						27	71	Fingers
14	22	Tail gunner	20	−40°C.	Oxygen mask froze; Wiring burned out; Gloves failed to heat	1 hour	Anoxia		109	Fingers
15			27.5	−48°C.					1 (year)	Fingers
16										Fingers, cheeks
17		Turret gunner	25	−32°C.	Urinated in suit		Previous frostbite			Buttock
18		Waist gunner	26	−40°C.	No heated clothes					Fingers
19		Waist gunner								Fingers
20		Waist gunner			Oxygen mask froze; Removed gloves	1 minute	Anoxia			Fingers, face

preserved. The largest groups of viable cells were at the apices of the rete pegs in the stripped-off epidemis (Fig. 5). Fibrinoid material lay amidst the loosened epithelium. Some cells were vacuolated or dyskeratotic and had enlarged bizarre nuclei; multinucleated syncytial giant cells were prominent (Fig. 6). There was precipitated material in the lumina of the sweat glands, and chromatin débris was present in the degenerated lining epithelial layer (Fig. 7). Even in better preserved glands the cells were swollen.

Necrotizing changes were prominent in the subcutaneous vessels, especially those in the fat lobules. The arterioles and venules had homogenized, hyalinized, smudgy walls, in which erythrocytes and bits of chromatin were embedded (Fig. 8). The nuclei of the degenerated endothelial cells were pyknotic or fragmented. Packed masses of erythrocytes and hyaline material plugged the small vessels; the red cells appeared conglutinated. The damaged vessels were not necessarily those most tightly crowded with erythrocytes. A scattering of leukocytes infiltrated the adipose tissue (Fig. 7), but the panniculitis was not accompanied by inflammation of the overlying dermis or of the adjacent fibrous septa.

Late Lesions

The description of the late lesions is a composite of observations made on material obtained for biopsy or at operation 2 to 50 weeks after exposure. Except for the vascular alterations, the histologic changes varied little from specimen to specimen.

With severe injury gangrene of the peripheral portion of the finger involved the deep structures as well as the superficial tissues. Necrosis was restricted to the epidermal roofs of vesicles when the patient had incurred only mild damage.

In mummified tissues the collagen appeared desiccated; the vessels were filled with homogenized masses of hemolyzed red cells. Bacteria had invaded the necrotic tissues, and colonies of organisms were particularly evident along the course of the hair follicles. There was extensive cellulitis in regions adjacent to areas of gangrene.

Both hyperplasia and atrophy of the epidermis were evident at the line of demarcation between gangrenous and viable portions of the finger and at the edges of ulcerated regions. In places a regenerated thin epithelial layer without pegs covered previously denuded areas, and pustular crusts often overlaid a subepithelial zone of inflamed granulation tissue (Fig. 9).

The sweat glands showed a variety of degenerative changes. Vacuolation of the epithelial cells and dilatation of the coils gave a microcystic appearance to some glands. Inspissated secretion filled a few

ducts, and the linings of others showed squamous metaplasia. Thickened basement membranes surrounded atrophic coils, and the fibrofatty lobules about the sweat glands showed depletion of fat and mucohyaline degeneration of the connective tissue. There were occasional cystic epidermal inclusions which may have arisen from sweat ducts. Inspissated desquamated material in some of them had provoked a granulomatous reaction in which foreign body giant cells were prominent.

There was diffuse sclerosis, particularly in older lesions; in some instances the subepithelial fibrosis resembled that of a keloid (Fig. 10). Usually there was a moderate infiltration by a variety of cells, but in one case a peculiarly even distribution of large mononuclear elements throughout the tissues was encountered (Fig. 16). Fibrosis and hyalinization often involved nerves, and there was perineural sclerosis (Fig. 11). Panniculitis, necrosis of fat, and lipoid phagocytosis were observed. Mucinous connective tissue replaced some adipose lobules, and in others the fat was atrophic and scarred (Fig. 12). Here and there scattered fat cells or a focal lipogranulomatous reaction marked the site of destroyed adipose tissue.

The evolution of the lesions was most clearly reflected in the progressive vascular changes; thrombosis, organization of thrombi, and obliterative angiitis were all encountered. The thrombi, which were of the agglutinative erthrocytic variety, contained little, if any, fibrin. They persisted in large vessels for as long as 6 weeks after exposure (Fig. 13), but only organized and recanalized plugs were encountered in fingers amputated after that interval (Fig. 14). Even in the organized thrombi clumps of well preserved erythrocytes and hemosiderophages were evident. The single or multiple new channels in the recanalized vessels sometimes had well developed musculo-elastic coats (Fig. 15). Some nonthrombosed arteries exhibited loose mucinous or lamellated fibrous thickening of the intima. There were also proliferative endophlebitic changes. The vessels coursing through relatively normal tissue, as much as 2 cm. above the zone of gangrene, were sclerotic. Scattered macrophages laden with hemosiderin marked the sites of old hemorrhages; they were often concentrated about vessels and nerves. Occasional vessels had mural hemorrhages. Chronic lesions exhibited many thin-walled collateral vascular channels (Fig. 16).

COMMENT

The lesions of high-altitude frostbite, like those of trench foot, are characterized by the prominence of thrombosis and vascular occlusion. The peripheral gangrene must be attributed to ischemia rather than to direct freezing of the tissues. The necrotizing vascular changes in the

earliest lesion bore no unequivocal relation to thrombotic plugs; they resembled the changes of perniosis and may have represented a direct effect of cold.

Although the thrombi are clearly agglutinative, there is no satisfactory explanation for the conglutination of red cells. In a study of experimentally produced frostbite, Lange [8] observed *in vivo* clumping of erythrocytes which could not be accounted for by loss of fluid from the vessels. In an earlier report he and his co-workers [9] pointed out that the clumps of red cells in the vessels could be washed free during the first 72 hours after exposure to cold but then formed cohesive thrombi. They discovered that heparinization prevented both thrombosis and gangrene.

Involvement of the adipose tissue was less in high-altitude frostbite than in trench foot, but the suggestion, previously advanced,[10] that cold has a special action on tissues rich in lipoid has received additional support from other sources. Two cases of pulmonary fat embolism following exposure to cold have been studied,[11, 12] and an example of crystallization of fat in an early lesion of trench foot has been encountered since the report on trench foot was published.

The material was not suitable for study of the sympathetic nerves. In the lesions of trench foot there was relative sparing of the vasoconstrictor fibers in mixed nerve trunks,[10] an observation at variance with that made by Blackwood in a study of immersion foot.[13] He noted damage to all fibers, especially the smaller and unmyelinated ones, although his observations, unfortunately, were erroneously reported in the paper on trench foot.[10] Blackwood and Russell [14] described degeneration of nerve and muscle, in the absence of vascular changes, following experimental exposure to cold.

High-altitude frostbite, a variety of "true frostbite," [5a, 15] is a classic example of the injury produced by "freezing," and trench foot exemplifies that caused by "chilling." The fact that no significant differences were noted between the lesions of high-altitude frostbite and those of trench foot [10] supports the concept that the reactions of tissues to cold exhibit a fundamental unity.[16] The differentiation between "chilling" and "freezing" injuries is becoming less sharp. For example, Lake [17] stated that even in cases of true frostbite the major part of the reaction may be of the secondary neurovascular type which follows chilling. Even Ungley, Channell, and Richards,[18] who wrote, "There has been a tendency to confuse the condition [immersion foot] with frostbite. This confusion persists even at the present day," admit, in the same paper, that "Cases of this nature are exceptional, but they suggest that the distinction between frostbite and immersion foot may not be so clear-cut as has been believed heretofore."

The superficial dissimilarities between the changes induced by freezing and those resulting from chilling reflect no fundamental difference in pathogenesis. The extreme degree of cold which causes frostbite, even if it acts for only a brief period, results in sharply demarcated and uniform damage. Prolonged exposure to a lesser degree of cold permits secondary factors, such as differences in sensitivity of tissues and local metabolic and vascular conditions, to exert their effects; as a consequence the reaction is unevenly distributed and irregularly demarcated. The results of varying exposures to different degrees of cold are roughly comparable to those produced by varied exposures to diverse amounts of radiant energy, another injurious physical agent. A necrotizing dose of irradiation will destroy all tissue within the field, while a smaller dose is followed by a complex of irregularly distributed destructive and reactive lesions, in the development of which secondary factors play important rôles.[19]

SUMMARY

Study of the frostbite incurred by aviators during bombing missions at high altitudes disclosed that the fingers were the most commonly damaged structures, although injury to the toes, face, and buttock was also encountered. The most prominent features of the morphologic changes were agglutinative thrombosis and vascular lesions. Gangrene probably resulted from ischemia, not from freezing. The similarity of the lesions to those of trench foot supports the view that the reactions of tissues following various types of exposure to cold exhibit a fundamental unity.

REFERENCES

1. Wells, H. V. Some aeroplane injuries and diseases, with notes on the aviation service. *J. Roy. Nav. M. Serv.*, 1916, 2, 65–71.
2. Cottet, J. Trench foot (etiology—pathology—symptomatology). *War Med.*, 1918–19, 2, 707–711.
3. Ducuing, J., D'Harcourt, J., Folch, A., and Bofill, J. Les troubles trophiques des extrémités produits par le froid sec en pathologie de guerre. *J. de chir.*, 1940, 55, 385–402.
4. Trench foot: special article prepared in the Office of the Surgeon General. *Army M. Bull.*, 1944, No. 74, 46–48.
5. Davis, L., Scarff, J. E., Rogers, N., and Dickinson, M. High-altitude frostbite; preliminary report. *Surg., Gynec. & Obst.*, 1943, 77, 561–575.
5a. Greene, R. Abstract of article by Davis, et al.[5] *Bull. War Med.*, 1943–44, 4, 573–574.
6. Cade, S. War surgery in the Royal Air Force. *Brit. J. Surg.*, 1944–45, 32, 12–24.
7. Sheeley, W. F. Frostbite, Eighth Air Force. *Air Surgeon's Bull.*, 1945, 2, 23–25.
8. Lange, K. Personal communication.
9. Lange, K., Boyd, L. J., and Loewe, L. The functional pathology of frostbite and the prevention of gangrene in experimental animals and humans. *Science*, 1945, 102, 151–152.

10. Friedman, N. B. The pathology of trench foot. *Am. J. Path.*, 1945, 21, 387–433.
11. Weller, C. V. Personal communication.
12. Mahon, H. M. Personal communication.
13. Blackwood, W. Injury from exposure to low temperature: pathology. *Brit. M. Bull.*, 1944, 2, 138–141.
14. Blackwood, W., and Russell, H. Further experiments in study of immersion foot. *Edinburgh M. J.*, 1945, 52, 160–165.
15. Safford, F. K., Jr., and Nathanson, M. B. Clinical observations on tissue temperatures; pathologic and therapeutic effects. *Arch. Surg.*, 1944, 49, 12–22.
16. Brownrigg, G. M. Frostbite; classification and treatment. *Am. J. Surg.*, 1945, 67, 370–381.
17. Lake, N. C. Frost-bite and Trench Foot. In: Bailey, H. (ed.). Surgery of Modern Warfare. Williams & Wilkins Co., Baltimore, 1942, ed. 2, 2, 530–547.
18. Ungley, C. C., Channell, G. D., and Richards, R. L. The immersion foot syndrome. *Brit. J. Surg.*, 1945–46, 33, 17–31.
19. Friedman, N. B. Effects of radiation on the gastrointestinal tract, including the salivary glands, the liver and the pancreas. *Arch. Path.*, 1942, 34, 749–787.

DESCRIPTION OF PLATES

PLATE 29

FIG. 1. Case 17. Frostbite of buttock.

FIG. 2. Case 2. Frostbite of fingers. Bullae, 2 days after exposure.

FIG. 3. Case 4. Frostbite of fingers. Gangrene, 15 days after exposure.

FIG. 4. Case 1. Frostbite of fingers. Gangrene, 15 days after exposure.
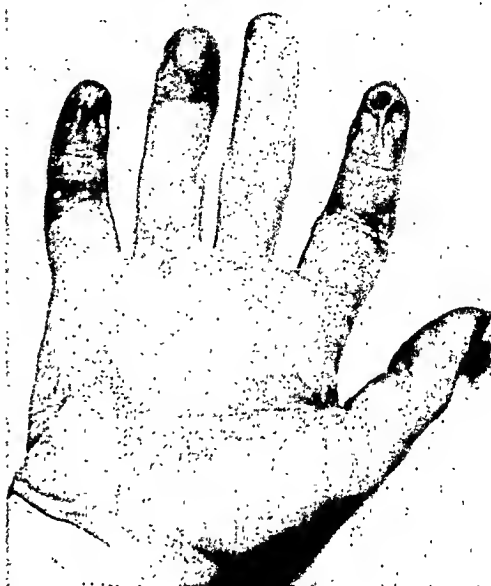
Friedman and Kritzler

High-Altitude Frostbite

PLATE 30

Early lesions, 3 days after exposure; biopsy specimen (case 1).

FIG. 5. Epidermal roof of vesicle. Preservation of epithelial cells at tips of pegs. × 105.

FIG. 6. Epidermal roof of vesicle. Bizarre and multinucleated epithelial elements. × 400.

FIG. 7. Degeneration of sweat glands. Leukocytic infiltration of adipose tissue. × 230.

FIG. 8. Necrosis of vascular walls. × 400.

PLATE 30

5

6

7

8

Friedman and Kritzler

High-Altitude Frostbite

PLATE 31

Friedman and Kritzler

High-Altitude Frostbite

13

14

15

16

Friedman and Kritzler

High-Altitude Frostbite

TUBEROUS SCLEROSIS WITH CONGENITAL TUMORS
OF HEART AND KIDNEY

REPORT OF A CASE IN A PREMATURE INFANT *

H. R. PRATT-THOMAS, M.D.

(From the Department of Pathology of the Medical College of the State of South Carolina, Charleston, S.C.)

The complex of tuberous sclerosis is of particular interest to pathologists and embryologists as it provides a striking example of the possible relationship between faulty cellular development and the process of neoplasia. The purpose of this paper is to describe the occurrence of tuberous sclerosis and other developmental defects in a premature infant of approximately 8 months' gestation who died 3 days after birth. This anomaly was associated with multiple rhabdomyomatous nodules of the heart and adenomatous foci in the kidneys. In addition, one of the nodules in the brain had assumed a distinctly neoplastic character. The cardiac neoplasms were of the type described by Batchelor and Maun [1] as "congenital nodular glycogenic tumors" and this instance is the 64th of this condition to be reported. Although tuberous sclerosis with or without malformations or tumors in other organs has been described in infants and young children by Globus and Selinsky,[2] Stewart and Bauer,[3] Farber,[4] Yater,[5] Hueper,[6] Labate,[7] Hillman,[8] Stewart,[9] and others, in so far as I can determine, this is the youngest infant in whom these changes have been observed.

REPORT OF CASE

A Negro girl, weighing 3 lbs. 15½ oz. and measuring 18 inches in length, was born on February 14, 1944. The mother, 19 years of age, had had swelling of hands and feet and headache during the last months of pregnancy. In the hospital the diagnosis of toxemia of pregnancy was made in view of a blood pressure of 165/110 mm. Hg, 2 plus edema of the lower extremities, and 4 plus albumin in the urine. It was her first pregnancy. Wassermann and Kline tests of the mother's blood were negative. The delivery was normal. The baby was thought to be in fair condition at birth and breathed spontaneously. Slight cyanosis was noted subsequently, but her condition did not become alarming until February 17th, when the cyanosis became marked and breathing difficult and gasping. The temperature rose to 100.5° F. and the baby died during the afternoon of the third day.

Gross Examination

The body was that of a small, poorly developed Negro baby girl weighing 1655 gm. No abnormalities of the skin or nails were noted.

The heart lay free within the pericardial sac and showed a smooth, round, button-like tumor projecting from the apex (Fig. 2). This mass measured 2.5 cm. across and 2.2 cm. in its superior-inferior

diameter. When viewed from the side the nodule was somewhat triangular, measuring 1.4 cm. in thickness where its base was attached to the heart, and only 0.3 cm. at its apex. Two smaller nodules were present about the base of the main mass and a separate nodule, 2 mm. in diameter, projected from the lateral aspect of the right ventricle. Within the right atrium a subendocardial nodule, 1 mm. in diameter, was located just above the attachment of the tricuspid valve. On section these nodules were composed of reddish tan tissue of nearly the same appearance as the myocardium but of lighter color. They were well delineated.

The lungs were congested and contained irregularly distributed, firm, subcrepitant areas.

Stripping of the renal capsules revealed prominent fetal lobulations. Visible on their surfaces were reddish gray areas, measuring from 1 to 4 mm. in diameter. They did not project above the surface but were imbedded in the periphery of the cortices.

The outstanding abnormality of the brain to be noted on external examination was a hard nodule in the tip of the right frontal lobe. The convolutions over the nodule were distorted, broad, and blunt. The mass measured 3 by 1.5 by 3 cm. and on section consisted of firm reddish gray tissue that was well demarcated from the adjacent brain substance (Fig. 1). The cerebral convolutions presented no other definite abnormality on inspection, but on palpation the surfaces of the cerebral hemispheres revealed nodular areas that were firmer than the adjacent soft cerebral tissue. A glistening gray nodular mass, measuring 1.2 cm. in greatest diameter, projected into the anterior horn of the lateral ventricle just medial to the left caudate nucleus (Fig. 2). A slight nodular elevation of the cerebral tissue was also visible in a similar position on the opposite side. Scattered through the brain, but particularly in the regions of the basal ganglia, were firm, nodular, grayish white areas. One well delineated nodule, measuring 0.5 cm. in diameter, was found close to the surface of the right occipital lobe. The other areas which imparted a firm nodular quality to the palpating finger showed no macroscopic abnormality.

Microscopic Examination

Heart. The myocardial tumors presented a markedly vacuolated appearance, identical with that of many of those previously described in the literature. The huge cells were usually rounded or ovoid, but often had crinkled cell boundaries. The cytoplasm of some was completely clear, but more often was finely granular or reticulated, and many of the clear cells had a thin rim of pale-staining cytoplasm. The nuclei, when present, varied between central and peripheral positions.

No mitotic figures were found. Striated myofibrils were situated between and along the borders of the large cells and often radiated from a central nucleus, so as to form the so-called spider cells (Fig. 3). The cross striations were accentuated with the aid of trichrome staining methods. The larger tumor nodules were sharply demarcated from the adjacent myocardium, but some of the smaller ones blended imperceptibly with the normal myocardial fibers. As many as eight nodules, only three of which were visible to the naked eye, were found in one section, measuring 1 by 0.5 cm. Nodules varied in size from the large tumor at the apex of the heart to tiny foci consisting of only three or four large vacuolated or granular cells. Best's carmine stain showed red glycogen granules in the cytoplasm of some of the cells, even after preservation of the heart in formalin for 18 months.

Kidney. In the peripheral portions of the renal cortex were large, dilated, tubular structures, usually arranged in groups of from three to twenty, but occasionally singly. These were lined by large cuboidal and columnar epithelial cells, sometimes in a single layer, but frequently several layers in thickness and often piled up and projecting into the lumina in papillary fashion (Fig. 4). Their cytoplasm was generally granular, but sometimes nearly clear. The cells contained granules of golden brown pigment as did the normal tubular epithelium. No mitotic figures were found.

Brain. The cytologic changes in the brain were essentially of two types, although variants of these occurred. The rather sharply defined nodules in the paraventricular regions and in the right frontal lobe differed chiefly in the degree of activity. They consisted of dense interlaced and whorled bundles of glial cells (Figs. 5 and 6), a majority of which were spongioblasts of unipolar or bipolar types (Fig. 7). Many large, bizarre and malformed cells of astrocytic type were present also (Fig. 8). These were frequently collected into groups and were particularly numerous about the periphery of the tumor of the frontal lobe. The cells in the paraventricular nodules tended to be more mature and no mitotic figures were found, whereas mitotic figures were easily found within the tumor of the frontal lobe.

The well defined nodules were well vascularized, the frontal tumor showing numerous engorged, thin-walled, branching, vascular channels of embryonal pattern. Foci of calcification were common and the transition from early degenerative changes to complete calcification could be traced in many of the enlarged misshapen cells. Nerve fibers and neurofibrils were demonstrable throughout the tumor nodules (Fig. 9). In the nodules and sclerotic patches myelination was generally poor and often practically absent.

In the tuberous nodules elsewhere there was gliosis consisting of

fully differentiated cells of astrocytic variety associated with varying numbers of large, rounded, ovoid, stellate, or globular cells (Fig. 10). These showed degenerative changes of which displacement of the nucleus, poor staining quality, hyaline degeneration of the cytoplasm, and great variation in the size, arrangement, and distribution of the processes were the most conspicuous. Some of these were definitely ganglion cells, but many appeared to be of glial origin. Differentiation between glial cells and nerve cells, however, was impossible in many instances, even with the aid of special gold and silver staining methods. In the sclerotic nodules the cyto-architecture was invariably severely deranged. Occasional vessels in the striate bodies were surrounded by collars of neuroblasts which extended into the adventitial coats. Petechial hemorrhages, often perivascular, were present in the internal portions of the cerebral hemispheres.

Lungs. The lungs showed intense congestion, with areas of early pneumonic exudation of lobular distribution.

DISCUSSION

Tuberous sclerosis and various tumors, notably cardiac rhabdomyomatous nodules, coexist so frequently that their relationship is surely more than fortuitous. This relationship strongly supports the theory concerning the histogenesis of these conditions, namely, that they are manifestations of a generalized developmental defect, probably due to defective cell potencies. It is for this reason that the term tuberous sclerosis complex has arisen. In the survey of 63 congenital "rhabdomyomas" of the heart compiled by Batchelor and Maun,[1] tuberous sclerosis was present in 32 cases, but they, as well as others, are of the opinion that the coexistence of these two lesions occurs in a much higher percentage of the cases. Of these 32, only 2 presented solitary cardiac tumors, thus emphasizing the greater tendency for multiple nodules to be associated with tuberous sclerosis.

Hueper[6] has called attention to the rarity of rhabdomyomatosis of the heart in the Negro. He described its occurrence in a Negro boy, and, of the 45 cases reported up to 1935, his was the only example in a Negro. These cardiac tumors, in contrast to congenital developmental disturbances in other organs, apparently never show malignant transformation.

Moolten,[10] Bielschowsky,[11] Globus, Strauss, and Selinsky,[12] and Globus,[12] and Ferraro and Doolittle[13] have called attention to the frequency with which actual neoplasms occur in the cerebral foci and take the form of a malignant mixed tumor (neurospongioblastoma). Globus,[14] in his discussion of primary neuro-ectodermal tumors of the

brain, described 12 cases of glioneuroma and 10 cases of spongioneuro-blastoma, with 2 of the former and all of the latter revealing features which indicated their close relationship to either fully developed or abortive forms of tuberous sclerosis. In practically every case of the glioneuroma group there were various cellular accumulations or patterns which suggested remnants of different phases of embryonal brain development.

Globus[14] felt that the presence of both spongioblastic and neuro-blastic derivatives gave support to the Cohnheim-Ribbert theory of origin of neoplasms in embryonal rests.

Various renal hamartomatous lesions are present in more than half of the patients having the tuberous sclerosis complex.[10] These lesions generally show an organoid structure, and true neoplasms are rare. The collections of abnormal tubules in the present case certainly suggest a hamartial defect rather than a true neoplasm.

CONCLUSION

A premature Negro infant showed well developed lesions of the tuberous sclerosis complex. The changes in the brain were far advanced and at least one of the nodules showed a truly neoplastic transformation (neurospongioblastoma). The cardiac tumors were of the type most commonly referred to as "congenital rhabdomyoma." This is the 64th example of that condition to be recorded.

REFERENCES

1. Batchelor, T. M., and Maun, M. E. Congenital glycogen tumors of the heart. *Arch. Path.*, 1945, 39, 67–73.
2. Globus, J. H., and Selinsky, H. Tuberous sclerosis in the infant. *Am. J. Dis. Child.*, 1935, 50, 954–965.
3. Stewart, H. L., and Bauer, E. L. Tuberous sclerosis. *Arch. Path.*, 1932, 14, 799–809.
4. Farber, S. Congenital rhabdomyoma of heart. *Am. J. Path.*, 1931, 7, 105–130.
5. Yater, W. M. Tumors of the heart and pericardium. Pathology, symptomatology and report of nine cases. *Arch. Int. Med.*, 1931, 48, 627–666.
6. Hueper, W. C. Rhabdomyomatosis of the heart in a Negro. *Arch. Path.*, 1935, 19, 372–379.
7. Labate, J. S. Congenital rhabdomyoma of the heart. Report of a case. *Am. J. Path.*, 1939, 15, 137–150.
8. Hillman, R. W. Tuberous sclerosis with congenital rhabdomyomas of the heart discovered in a child dying of acute lead poisoning. *Brooklyn Hosp. J.*, 1941, 3, 181–195.
9. Stewart, D. T. Rhabdomyomatosis of the heart associated with tuberose sclerosis of the brain and multiple cysts of the kidney. *New Zealand M. J.*, 1939, 38, 19–22.
10. Moolten, S. E. Hamartial nature of the tuberous sclerosis complex and its bearing on the tumor problem; report of a case with tumor anomaly of the kidney and adenoma sebaceum. *Arch. Int. Med.*, 1942, 69, 589–623.

11. Bielschowsky, M. Zur Histopathologie und Pathogenese der tuberösen Sklerose. *J. f. Psychol. u. Neurol.*, 1924, 30, 167–200.

12. Globus, J. H., Strauss, I., and Selinsky, H. Das Neurospongioblastom, eine primäre Gehirngeschwulst bei disseminierter Neurospongioblastose (tuberöse Sklerose). *Ztschr. f. d. ges. Neurol. u. Psychiat.*, 1932, 140, 1–29. Globus, J. H. Neurinome central associé à une sclérose tubéreuse (neurospongioblastose disséminée). *Rev. neurol.*, 1933, 2, 1–24.

13. Ferraro, A., and Doolittle, G. J. Tuberous sclerosis (diffuse neurospongioblastosis). *Psychiatric Quart.*, 1936, 10, 365–416.

14. Globus, J. H. Glioneuroma and spongioneuroblastoma, forms of primary neuroectodermal tumors of the brain. *Am. J. Cancer*, 1938, 32, 163–220.

DESCRIPTION OF PLATES

PLATE 33

FIG. 1. Cross section of the brain showing the tumor of the frontal lobe and nodular masses projecting into the ventricle.

FIG. 2. Rhabdomyomatous nodule projecting from the apex of the heart.

FIG. 3. Large, clear, granular and vacuolated cells of the cardiac tumor, including spider cells. Pollak's modification of the trichrome stain. × 365.

PLATE 34

FIG. 4. Dilated renal tubular structures lined by layers of cuboidal and columnar epithelium. Hematoxylin and eosin stain. × 115.
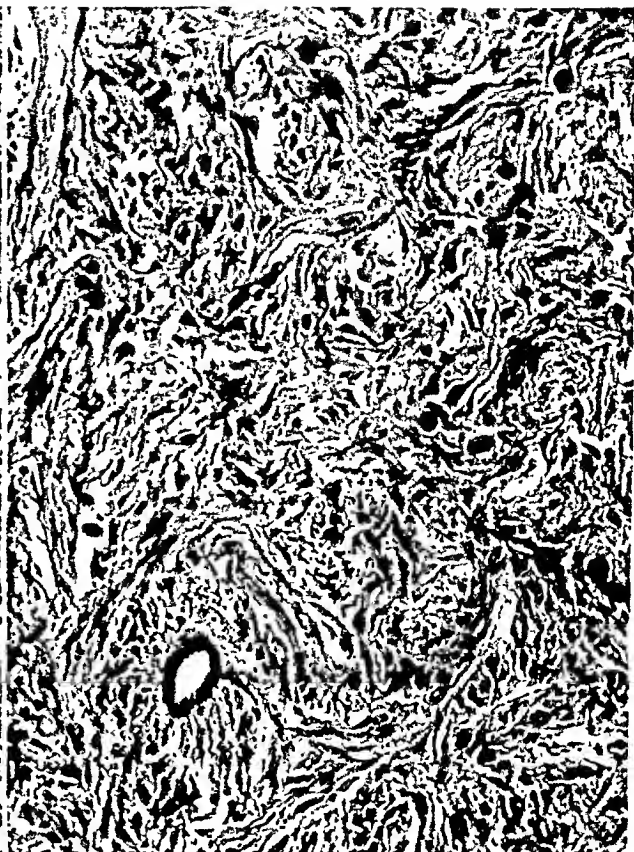
FIG. 5. Interlacing bundles of glial fiber in a ventricular nodule. Globus' modification of the gold sublimate stain. × 115.

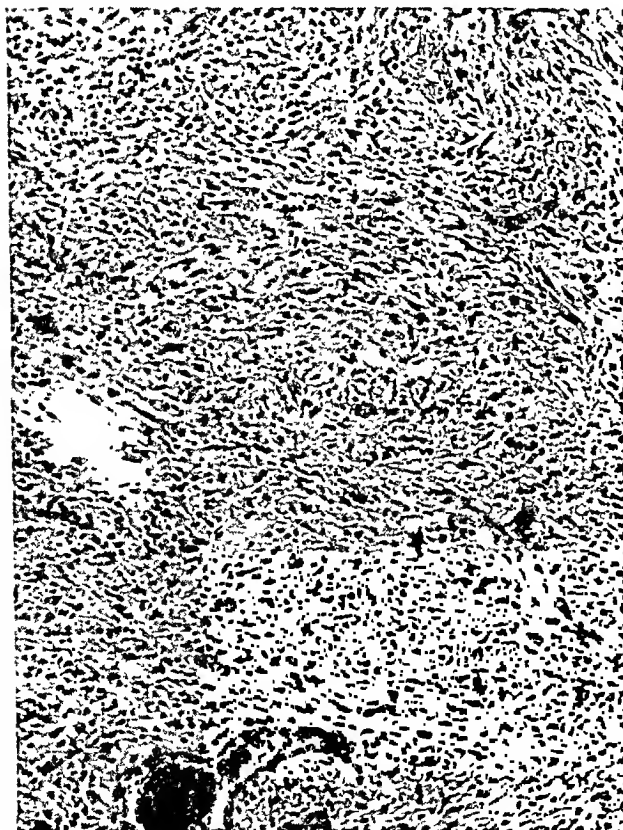FIG. 6. The tumor of the frontal lobe. Hematoxylin and eosin stain. × 115.

FIG. 7. Partially differentiated spongioblasts in the tumor of the frontal lobe. Globus' modification of the gold sublimate method. × 385.
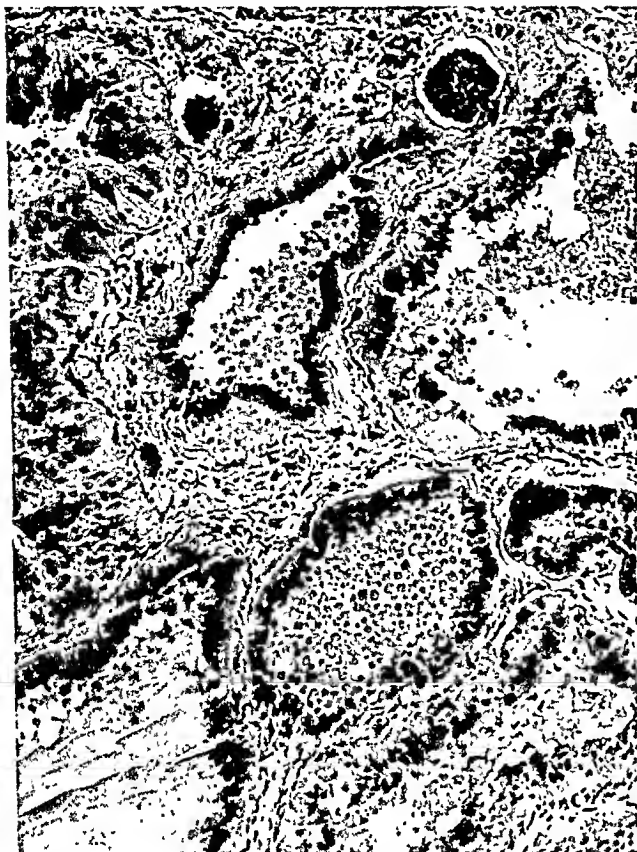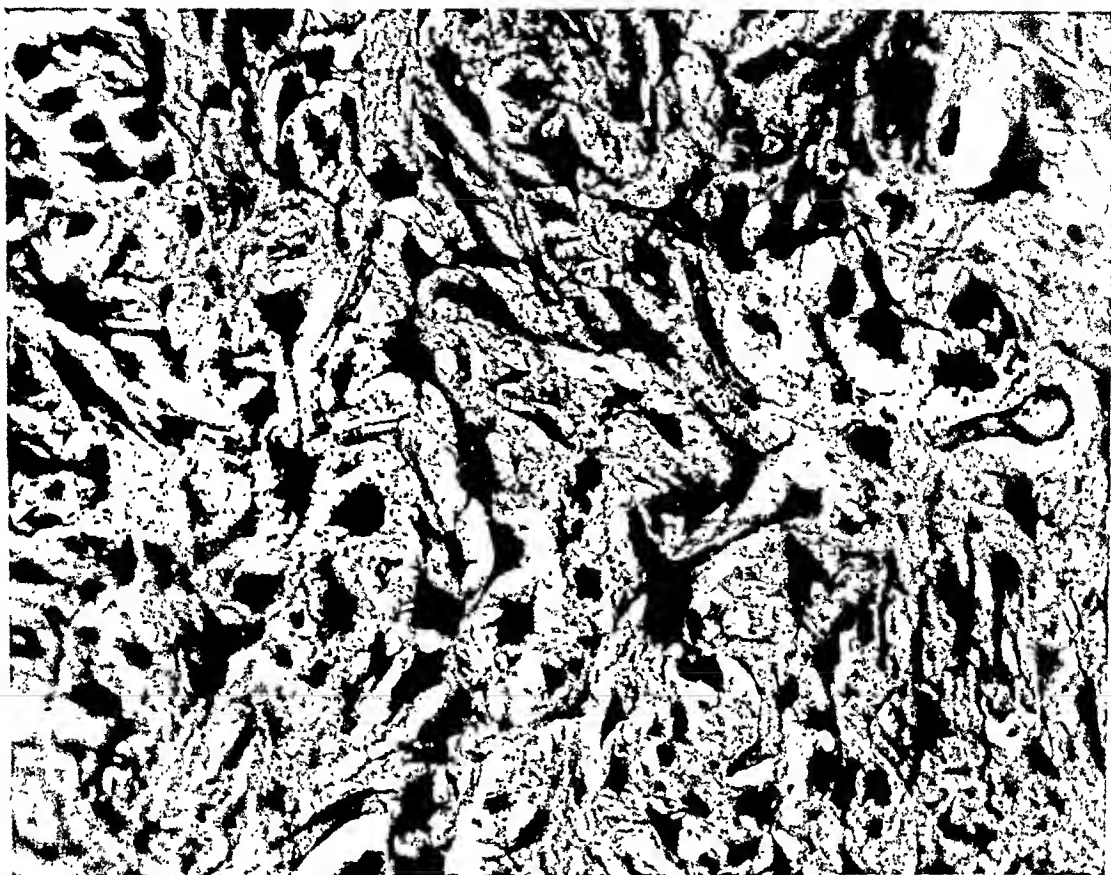
4

5

6

7

Pratt-Thomas

Tuberous Sclerosis

PLATE 35

FIG. 8. Bizzare, atypical glial elements in the periphery of ventricular nodules. Globus' modification of the gold sublimate method. ✕ 350.

FIG. 9. Nerve fibrils among abnormal misshapen cells of the tumor of the frontal lobe. Bodian's method. ✕ 600.

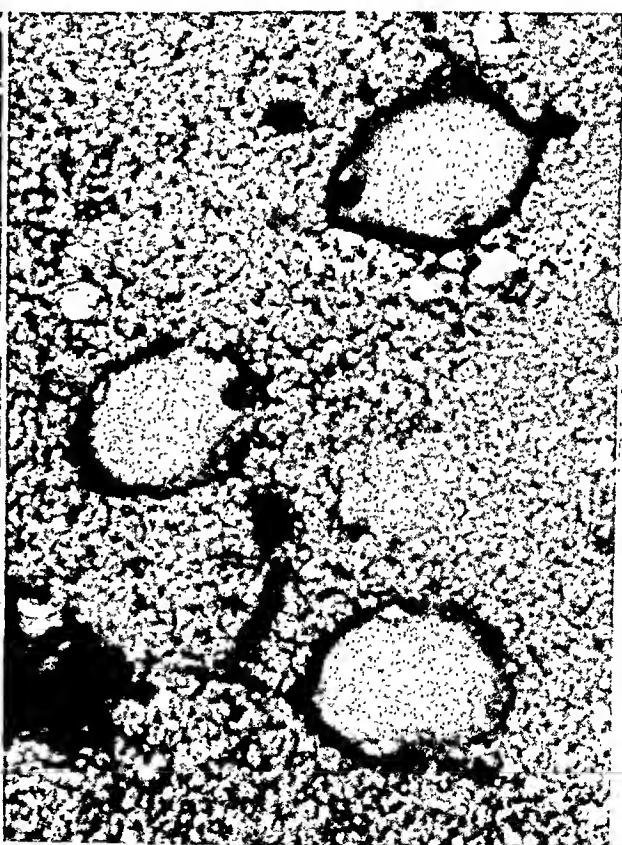FIG. 10. Large, degenerating balloon-shaped cells in sclerotic nodules. Cresyl violet stain. ✕ 600.

8

9

10

THE PATHOGENESIS OF POLYCYSTIC LIVERS

RECONSTRUCTIONS OF CYSTIC ELEMENTS IN TWO CASES *

ROBERT F. NORRIS, M.D.,† and RALPH M. TYSON, M.D.

(*From the Ayer Clinical Laboratory and the Pediatric Service, Pennsylvania Hospital, Philadelphia, Pa.*)

The occurrence of congenital polycystic disease of the liver has been known and studied for a long time and the cause of the lesions has been the subject of much speculation. In addition to numerous case reports, reviews of the literature and discussions of the problem of etiology may be found in the papers of Kahlden,[1] Still,[2] Moschcowitz,[3] Bunting,[4] Vorpahl,[5] Meyenburg,[6,7] Sears,[8] Teuscher,[9] Wackerle,[10] Delore and Croizat,[11, 12] Rümler,[13] Lutembacher,[14] Baccarini,[15] and Monserrat and Latienda.[16]

In those papers, it is clear that the cystic lesions have been thoroughly studied microscopically, often in serial sections, but they have not been evaluated by means of reconstructions. However, since it may rightly be assumed that the origin and mode of development of cystic lesions in all organs are governed by the same principles and since studies of models from polycystic kidneys have been made, the theories which have been proposed for the etiology of polycystic kidneys have been applied to polycystic disease of the liver. The kidney, furthermore, is probably the organ best suited for the study of this disease, since it is formed from two separated anlagen which subsequently unite and the individual nephrons are composed of elements from both anlagen. In the kidney, therefore, the character of developmental defects and the time of their occurrence should be much more easily demonstrated than in organs such as the liver, pancreas, and lungs, in which the proper formation of individual epithelial elements is not dependent upon the union of different anlagen. Nevertheless, information on these points has been only slowly accumulated. Consequently, the problem of polycystic disease of the liver will be more easily understood if the theories of origin of polycystic kidneys are briefly reviewed.

At first it was thought by Virchow,[17, 18] and others that inflammatory lesions of the fetal kidney might disturb the developing nephrons sufficiently to cause obstruction and cystic dilatation. This theory at present is not in favor since inflammatory lesions are not always present and could scarcely be hereditary. Later it was suggested by

Mutach,[19] Ribbert,[20] and others that failure of union of the two anlagen might explain these lesions. Although attractive by virtue of its simplicity, this theory is untenable since it does not explain why polycystic disease occurs in organs in which the union of two separate anlagen does not occur and since it has been shown recently [21,22] that the two anlagen in all probability do unite. Because of the large size of many cysts and the occasional finding of undifferentiated masses of epithelial cells, it was proposed by Brigidi and Severi,[23] and others that the lesions were truly neoplastic. There seems little basis for this argument since the cysts show no evidence of rapid proliferation and the great majority are lined by only a single layer of epithelium. Much more logically, Albrecht [24] suggested the term "Hamartome" for these lesions, which impressed him as being an improper proliferation of tissue as a consequence of faulty development. However, recently, doubt has been cast upon the fundamental importance of excessive proliferation of epithelium,[22] since in one case segmentation of nephrons occurred without excessive dilatation or proliferation of epithelium. Important contributions to the subject recently were made by Kampmeier,[25-28] and McKenna and Kampmeier,[29,30] These workers demonstrated that the first generations of nephrons in the metanephros are normally provisional and often persist for short periods in fetal life as small, isolated cysts. They concluded that persistence of these segments as cysts might explain the occurrence of polycystic disease. More recently, however, Norris and Herman [22] found in one of their cases that virtually all of the nephrons were segmented and cystic and that the total number of nephrons was approximately normal for a newborn child. By means of serial sections and reconstructions, 4 cases were studied, 2 of which were newborn infants and 2 adults. In the 2 infants, normally formed but isolated glomeruli without marked capsular dilatation were found in the cortex, and blindly-ending but undilated collecting ducts emptying into the calyces were found in the papillae. Between the two zones were cysts and undilated segments of nephrons. It was concluded, first, that for the glomeruli to be so perfectly formed, continuity between the nephrogenic anlage and ureteric anlage must first have been established and have been followed by segmentation and cystic dilatation of the detached elements and, second, that excessive epithelial proliferation was not necessarily responsible for the lesions. It was also pointed out from the literature that the mesonephros normally degenerates by a process of segmentation as do the early generations of nephrons in the metanephros. It was suggested, therefore, that polycystic disease of the kidneys is in reality an extension of this normal process of degeneration to include some or all of the later generations of nephrons.

To determine whether this last theory is also applicable to poly-cystic disease in general, study of other cystic organs is essential. For this purpose, we are reporting descriptions of cystic livers, together with reconstructions of some of the lesions, from the 2 infants previously reported by Norris and Herman.[22]

MATERIALS AND METHODS

Case numbers 1 and 2 of the present report correspond to the case numbers given before by Norris and Herman.[22] Briefly, case 1 was that of a full-term male infant in whom greatly enlarged polycystic kidneys and liver so embarrassed respirations that he died 20 minutes after birth. Case 2 was that of a full-term female infant who developed a hemorrhagic diathesis, jaundice, and evidence of renal insufficiency and died 24 days after birth. Following delivery, sixth digits of both hands were amputated. Relatively undilated cystic lesions of the kidneys, liver, and pancreas were found at autopsy. Since in the previous paper the clinical histories, anatomic diagnoses, and detailed findings in the kidneys were presented, only the gross and microscopic lesions of the livers will be described at this time.

All tissue was fixed either in Kaiserling's or Regaud's solutions. Blocks were embedded in paraffin and sections were stained with Delafield's hematoxylin and eosin. Sections for ordinary study were cut at 5 μ. From each liver, several hundred serial sections, 15 μ in thickness, were cut from each of four blocks about 2 cm. on a side. The cystic lesions and distorted bile ducts were traced and studied microscopically through these sections. Reconstructions were accomplished by the method previously described.[21]

OBSERVATIONS

Case 1

Gross Examination. The liver of case 1 weighed 270 gm. and, in spite of the enlargement, had distinctly normal contours. There were distinct right and left lobes. The gallbladder was partially distended with thick, dark green bile and, except for post-mortem changes of the mucosa, was normal. The cystic duct, extrahepatic bile ducts, and common duct were also patent and normal. On section, the parenchyma was bloody throughout. The lobular architecture was evident but was not as distinct as is normal. In the periphery of the lobules highly irregular cystic dilatations were seen averaging about 3 mm. in diameter. There were no large cysts nor any focal lesions.

Microscopic Examination. The lobular architecture of the liver was not distorted and branches of the hepatic veins were situated normally in the center of each lobule. The parenchymal liver cells showed no

lesions, but the sinusoids were slightly dilated and large areas of erythropoiesis and myelopoiesis were present. The periportal areas, however, were greatly distorted by marked cystic dilatations of the intrahepatic bile ducts which, as seen in low-power fields (Fig. 3), completely encircled cords of loose connective tissue containing branches of the hepatic artery and portal vein. These cysts were lined by single layers of cuboidal or low-columnar epithelium. The cytoplasm of these cells was usually clear and the nuclei were vesicular. There was no evidence of active proliferation. Under higher power, about the periphery of these cystic areas were seen numerous highly distorted bile ducts, lined by epithelium of similar character, which were not markedly dilated (Fig. 4). These, also, had a conspicuous tendency to encircle the branches of the portal vein and hepatic artery. The connective tissue surrounding these ducts was loose and in many areas cellular elements of the foci of hematopoiesis were migrating through the epithelium to the lumina of the ducts. Nearly all of the cystic bile ducts contained erythrocytes and casts of both hemoglobin and bile. The small bile canaliculi, however, were not dilated and did not contain casts.

In serial sections, as can be seen in the model (Fig. 1), the encirclement of the hepatic arteries and portal veins by the greatly dilated bile ducts was also readily demonstrated. The cystic ducts were highly irregular in contour and there were numerous large outpocketings. Anastomoses were numerous and, in many places, undilated but distorted ducts formed continuations and branches of the cystic ducts. Although many of the outpocketings or branches were blindly-ending, it was not demonstrated that any of the large dilated ducts were completely isolated as cysts. The courses of the hepatic arteries and portal veins were generally straight and uninterrupted although there was considerable irregularity of the contours of these vessels.

Case 2

Gross Examination. The liver of case 2, which weighed 220 gm., was normally formed and had distinct right and left lobes. The gall-bladder contained a small amount of yellow bile and, except for post-mortem changes of the mucosa, was normal. The cystic duct, extrahepatic bile ducts, and common duct were likewise patent and normal. On section, the lobular architecture of the liver was grossly normal, the intrahepatic bile ducts were not dilated, and no focal lesions were seen.

Microscopic Examination. In low-power fields, the lobules of the liver were generally normal in contour. The central veins were not

remarkable and the parenchymal cells and sinusoids showed no lesions. Many of the branches of the portal vein, however, appeared dilated and were surrounded by larger numbers of bile ducts than is normal (Fig. 5). Under higher power, these ducts, although not conspicuously dilated, were highly irregular in contour, appeared more numerous than is normal and were grouped around the branches of the portal vein and hepatic artery (Fig. 6). They resembled the nondilated ducts in case 1 (Fig. 4). Individually, the lining cells were low-columnar or cuboidal. The cytoplasm was clear and colorless and the nuclei were vesicular. There was no evidence of rapid cellular proliferation. Many of the small bile canaliculi and some of the small bile ducts were slightly distended with casts of inspissated bile. There was no extra-medullary hematopoiesis.

In serial sections, irregularity and distortion of individual bile ducts were marked. As can be seen in the reconstruction (Fig. 2), the ducts sometimes were narrow and sometimes were focally dilated. Small and large segments of ducts were often isolated as cystic structures without significant enlargement or dilatation when compared with the adjacent unsegmented ducts. Many of these isolated segments were in a direct line with branches of biliary ducts which were not segmented. This observation suggests that these segments were previously in continuity with the bile ducts and secondarily became isolated as nondilated cysts. Although the branches of the hepatic artery were generally straight, the branches of the portal vein were often distorted. No isolated segments of veins were identified.

DISCUSSION

In the cases presented, the conspicuous lesions were those of the small intrahepatic bile ducts. The ducts in both cases were highly irregular in contour and diameter and did not conform to a regular pattern. The number of ducts in the periportal areas appeared to be greater than is normal. This was especially true in case 2, as can be seen in the model (Fig. 2). Anastomoses in both cases were numerous. In case 1, many of the ducts were not dilated, but in nearly all of the periportal areas there were large cystic ducts which in places completely encircled the hepatic artery and portal vein. None of these, however, were demonstrated as being isolated, blindly-ending cysts. By contrast, in case 2 none of the ducts were greatly dilated but many small and large segments were isolated and were blindly-ending in both directions. That the flow of bile, elaborated by the parenchymal epithelial cells, was obstructed in many places was shown by jaundice and by bile casts in the canaliculi. In case 1, however, there was no jaundice and the

canaliculi did not contain bile casts, so that biliary obstruction was not demonstrated.

It is surprising that in case 1 the large cystic ducts were not found isolated as cysts. If these ducts were not segmented, as the lack of obstructive jaundice also indicates, then it may be argued that cystic dilatation may occur before segmentation and isolation of· these elements as cysts. In case 2, by contrast, segmentation occurred without cystic dilatation. On the other hand, in the kidneys of case 1 similar large cystic dilatations were shown definitely to be isolated as cysts. In the latter, there were also numerous anastomoses. It is quite possible, therefore, that similar anastomoses in the liver obscured a previous tendency to segmentation and isolation of cysts.

In the kidneys, although the method for estimating the number of nephrons was admittedly crude, it was not demonstrated that there was an overproduction of elements. In the livers, more than the usual number of bile ducts appeared to be present in the periportal areas. Theoretically, an excessive production of elements persisting until birth can occur, since early generations of nephrons are normally provisional as are many of the small intrahepatic bile ducts. The association of polydactylism with polycystic disease also suggests this possibility. That an overproduction of elements is significant in the pathogenesis of polycystic disease has been previously suggested and has been used as the basis for the assumption that abnormal proliferation of epithelial elements is the fundamental lesion of the disease.[13,23,24] However, the liver differs from the kidney in that bile ducts can regenerate in various diseases whereas nephrons, after birth, at least, do not have this potentiality. Moreover, anastomoses among small bile ducts are the rule but do not occur normally among nephrons. Consequently, so far as the present cases are concerned, preceding abnormalities of the developing bile ducts might stimulate the production of excessive numbers of elements. The apparent overproduction of bile ducts would then be the result of the lesions rather than the cause of them.

Before discussing further the significance of the cystic lesions which have been described and illustrated in these cases, it is important to review the normal development of the liver. According to Lewis,[31] the anlage of the liver is a median ventral outgrowth of the entodermal tube. Cords of epithelial cells proliferate distally and are later separated from the gut by a short, solid stem. Eventually this stem becomes canalized to form the common bile duct. Meanwhile the trabeculae and cords of proliferating epithelial cells indent the lumina of the omphalomesenteric veins which grow out between the cords to invest

and surround them with endothelium. The right omphalomesenteric vein later becomes the portal vein and the hepatic vein is essentially the persistent outlet of this right omphalomesenteric vein. The left umbilical vein also sends branches to the liver and becomes the round ligament after birth. For a period in embryonic development, the hepatic vein is connected with the portal and umbilical veins by a large blood sinus within the liver, the ductus venosus, which disappears before birth by subdividing into the sinusoidal circulation. Meanwhile, branches of the hepatic artery are proliferating along branches of the hepatic ducts which are developing in continuity with the common duct. The intrahepatic ducts, however, do not proliferate extensively until after branches of the portal vein are formed and generally follow the course of these branches. Between the embryonic stages of 10 and 23 mm., segments of blindly-ending ducts may be observed. These blend with the hepatic trabeculae, and it is not until the stage of about 23 mm. that proliferation of the duct system is active. These ducts form a plexus in the periportal mesenchyma. Anastomoses which are numerous at first are fewer at the time of birth, although fluid injected into one hepatic duct will be returned by way of the other.

From these facts concerning the development of the normal liver it is possible to date roughly the origin of the lesions in the present cases. Since intrahepatic bile ducts do not appear until the embryo is 10 mm. in length and do not proliferate actively until about 23 mm., 10 to 23 mm. is the earliest stage at which the lesions of the ducts could begin. By this time, however, the lobules are beginning to differentiate and branches of the portal vein and hepatic artery are already appearing in the perilobular mesenchyma. It is quite possible, therefore, that pathologic changes of the ducts did commence at this stage. The encirclement of branches of the portal vein and hepatic artery by dilated ducts in case 1 indicates progressive enlargement of the ducts after this initial stage and it is altogether likely that the development of the lesions in both cases was continuous but gradual.

The gross structure of the livers in both of the present cases was remarkably normal. The gallbladders were normally formed and there was no dilatation or atresia of the common bile ducts. Both grossly and microscopically, the lobular pattern was normal. The central veins, sinusoids, and parenchymal epithelial cells showed no lesions. Although there was some distortion and irregularity of the branches of the portal vein and hepatic artery, they were normally situated in the perilobular connective tissue. In both cases, therefore, the principal lesions were confined to the small intrahepatic bile ducts and were of such a nature that the normal development of the other elements of

the liver and biliary system was not prevented or significantly altered. This observation is also in agreement with the fact that the structural development of the cystic kidneys in those cases was likewise normal.

If these lesions were caused by inflammation, of which there was no evidence, or by an unrestrained proliferation of the bile ducts, it is difficult to understand how the rest of the liver could be so normal. Likewise, since so many of the ducts were distorted or segmented, the occurrence of the lesions can hardly be explained by the persistence of the few normally provisional bile ducts in accordance with Kampmeier's theory that persistence of normally provisional nephrons accounts for the polycystic lesions of the kidney.

In view of these various considerations, therefore, it is proposed that, as for the kidneys, differentiation of the hepatic anlage was at first normal. Only after the appearance of the small intrahepatic bile ducts did cystic lesions begin. Whether the apparent overproduction of the bile ducts was primary or secondary is immaterial. Distortion, segmentation, and cystic dilatation of these elements occurred progressively while the normal differentiation of the rest of the liver was proceeding uninterruptedly. Fundamentally, this is a process of degeneration and is analogous to the sequence of anatomic changes which have been demonstrated in the kidneys. As segmentation of elements is the initial stage of resorption in the mesonephros and in the first generations of nephrons in the metanephros, so the early generations of bile ducts normally segment before complete degeneration and resorption. Consequently, it is believed that in polycystic disease of the liver many more of the small intrahepatic bile ducts than is normal are provisional and that persistence of these segments, which may progressively become dilated as cysts, explains the polycystic disease of the adult. The evidence presented confirms the previous hypothesis that polycystic disease in general is occasioned by an abnormal extension of a normal process of degeneration. The lesions may or may not be accompanied by an overproduction of elements and cystic dilatation may occur before or after segmentation. The familial incidence of the disease strongly suggests a hereditary defect.

SUMMARY AND CONCLUSIONS

As part of a study of the polycystic lesions in the livers of 2 infants, three-dimensional reconstructions of some of the elements were prepared.

The lesions, confined exclusively to the intrahepatic bile ducts, consisted of distortion, segmentation, and dilatation.

The normal development and differentiation of the rest of the liver and biliary tract was not prevented or significantly altered.

As is the case in polycystic kidneys, it was concluded that the lesions in the livers are essentially degenerative and are an abnormal extension of the process of resorption which occurs normally in the first generations of bile ducts.

REFERENCES

1. Kahlden, C. Ueber die Genese der multiloculären Cystenniere und der Cystenleber. *Beitr. f. path. Anat. u. z. allg. Path.*, 1893, 13, 291–308.

2. Still, G. F. Congenital cystic liver with cystic kidney. *Tr. Path. Soc. London*, 1897–98, 49, 155–165.

3. Moschcowitz, E. Non-parasitic cysts (congenital) of the liver, with a study of aberrant bile ducts. *Am. J. M. Sc.*, 1906, 131, 674–699.

4. Bunting, C. H. Congenital cystic kidney and liver with family tendency. *J. Exper. Med.*, 1906, 8, 271–288.

5. Vorpahl, F. Über einen Fall von kongenitaler Cystenleber und Cystenniere mit Übergang in riesenhafte Kystombildung. *Beitr. z. path. Anat. u. z. allg. Path.*, 1912, 53, 477–490.

6. Meyenburg, H. v. Über Atresie der grossen Gallenwege. *Virchows Arch. f. path. Anat.*, 1916, 221, 352–366.

7. Meyenburg, H. v. Über die Cystenleber. *Beitr. z. path. Anat. u. z. allg. Path.*, 1917–18, 64, 477–532.

8. Sears, W. G. Congenital cystic disease of the kidneys, liver, and pancreas. *Guy's Hosp. Rep.*, 1926, 76, 31–52.

9. Teuscher, M. Über die kongenitale Cystenleber mit Cystennieren und Cystenpankreas. *Beitr. z. path. Anat. u. z. allg. Path.*, 1926, 75, 459–485.

10. Wackerle, L. Zur Frage der Cystenleber. *Virchows Arch. f. path. Anat.*, 1926, 262, 508–530.

11. Delore, P., and Croizat. Contribution à l'étude de la maladie polykystique. Un cas de maladie polykystique du foie et des reins avec porencéphalie et hémiplégie infantile. *Arch. franco-belges de chir.*, 1928, 31, 177–185.

12. Delore, P., and Croizat. Un caso di malattia policistica del fegato e dei reni con poroencefalia ed emiplegia infantile. (Contributo allo studio della malattia policistica.) *Rinasc. med.*, 1929, 6, 158–161.

13. Rümler, E. Die polycystische Entwicklungsstörung im Pankreas, zugleich ein Beitrag zur Frage der Cystenleber und der Cystennieren. *Virchows Arch. f. path. Anat.*, 1934, 292, 151–165.

14. Lutembacher, R. Reins polykystiques congénitaux à kystes miliaires. *Ann. de méd.*, 1936, 39, 440–448.

15. Baccarini, L. Contributo allo studio della patogenesi del "fegato policistico." *Arch. ital. di chir.*, 1936, 43, 92–114.

16. Monserrat, J. L., and Latienda, R. I. Riñon e hígado micropoliquístico (disembrioplasia micropoliquística hepato-renal). *Arch. Soc. argent. de anat. norm. y. pat.*, 1942, 4, 88–94.

17. Virchow, R. Über Hydrops renum cysticus congenitus. *Virchows Arch. f. path. Anat.*, 1869, 46, 506–507.

18. Virchow, R. Verhandlungen ärztlicher Gesellschaften. *Klin. Wchnschr.*, 1892, 29, 104–107.

19. Mutach, A. v. Beitrag zur Genese der congenitalen Cystennieren. *Virchows Arch. f. path. Anat.*, 1895, 142, 46–86.

20. Ribbert, H. Ueber die Entwicklung der bleibenden Niere und über die Entstehung der Cystenniere. *Verhandl. d. deutsch. path. Gesellsch.*, 1899, 2, 187–203.

21. Norris, R. F., and Herman, L. Observations on the anatomic basis of congenital polycystic kidneys. *Tr. Am. A. Genito-Urin. Surgeons*, 1938, 31, 41–57.

22. Norris, R. F., and Herman, L. The pathogenesis of polycystic kidneys: Reconstruction of cystic elements in four cases. *J. Urol.*, 1941, 46, 147–176.

23. Brigidi, V., and Severi, A. Contributo alla patogenesi delle cisti renali. *Sperimentale, Arch. di biol.*, 1880, 46, 1–25.

24. Albrecht. Ueber Hamartome. *Verhandl. d. deutsch. path. Gesellsch.*, 1904, 7, 153–157.

25. Kampmeier, O. F. Über das Schicksal der erstgeformten Harnkanälchen der bleibenden Niere beim Menschen. *Arch. f. Anat. u. Physiol., anat. Abteil.*, 1919, 204–226.

26. Kampmeier, O. F. A hitherto unrecognized mode of origin of congenital renal cysts. *Surg., Gynec. and Obst.*, 1923, 36, 208–216.

27. Kampmeier, O. F. Weitere Studien über die Entwicklungsgeschichte der bleibenden Niere beim Menschen. *Ztschr. f. Anat. u. Entwcklngsgesch.*, 1924, 73, 459–500.

28. Kampmeier, O. F. The metanephros or so-called permanent kidney in part provisional and vestigial. *Anat. Rec.*, 1926, 33, 115–120.

29. McKenna, C. M., and Kampmeier, O. F. A consideration of the development of polycystic kidney. *Tr. Am. A. Genito-Urin. Surgeons*, 1933, 26, 377–383.

30. McKenna, C. M., and Kampmeier, O. F. A consideration of the development of polycystic kidney. *J. Urol.*, 1934, 32, 37–43.

31. Lewis, F. T. The Development of the Liver. In: Keibel, F., and Mall, F. P. (eds.) Manual of Human Embryology. J. B. Lippincott Co., Philadelphia, 1912, 2, 403–428.

DESCRIPTION OF PLATES

PLATE 36

FIG. 1. Case 1. Model of dilated cystic bile ducts. Anastomoses and blindly-ending projections are numerous. At the extreme left of the model, relatively nondilated branches of the duct are seen in cross section. At the extreme right, the dark tubular structures are branches of the portal vein which in this instance lie outside the duct. Encompassed by the two large cystic ducts are branches of the hepatic artery which are also tubular and less darkly shaded. In the center of the model, windows have been cut to illustrate the cystic folds of the duct and the interior location of the artery. The approximate vertical extent of the reconstruction in the liver was 0.64 cm.

FIG. 2. Case 2. Model of reconstructed elements. Branches of the hepatic artery (A) and portal vein (V) are generally straight but are intimately associated and frequently surrounded by the numerous, more lightly shaded bile ducts. The ducts show considerable variation in diameter and lack a regular pattern. Many branches end blindly. In the lower half of the model are several completely isolated globular segments of ducts as well as a minute circular segment overlying the vein. Just to the right of center, also overlying a vein, is a highly irregular, nondilated segment of duct. Elsewhere there are several more isolated minute segments. Many of the isolated segments appear to be detached continuations of nearby ducts. The approximate vertical extent of the reconstruction in the liver was 0.14 cm.

PLATE 36

AMERICAN JOURNAL OF PATHOLOGY. VOL. XXIII
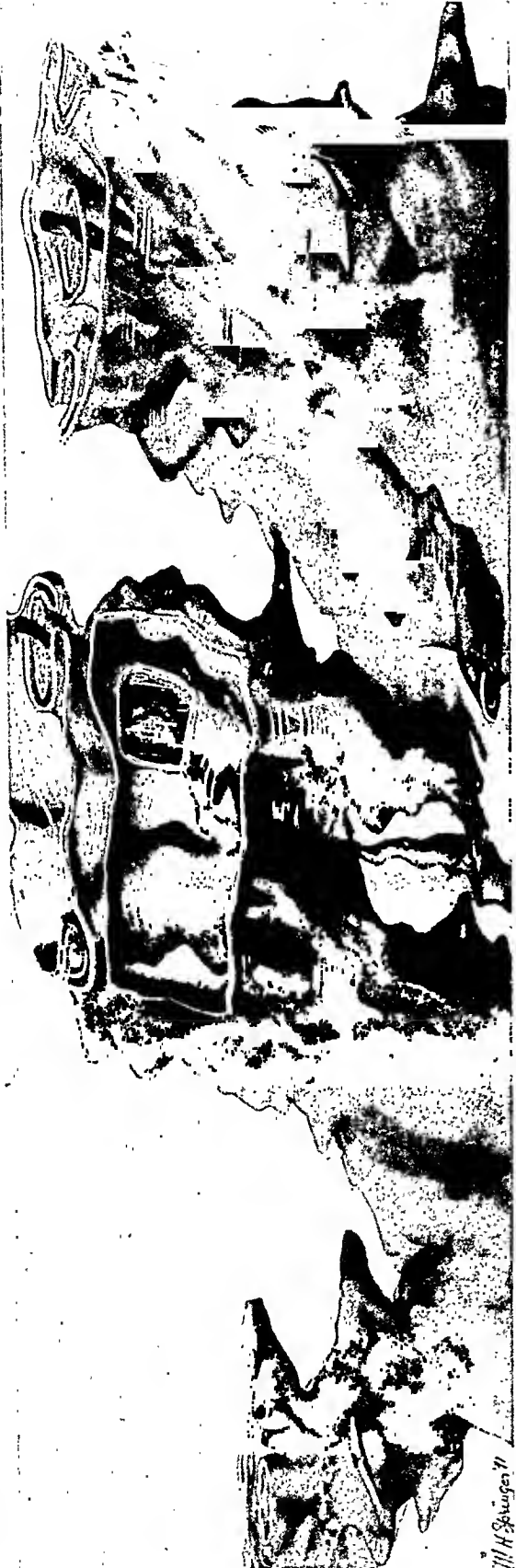
1

2

PLATE 37

FIG. 3. Case 1. Low-power photomicrograph showing a large cyst which is a cross
section of a dilated bile duct. The lining columnar epithelium is generally
detached. The circular cores of solid tissue within the lumen of the cyst are
cross sections of trabeculae composed of periportal connective tissue which
have been completely encircled and invested by the dilated duct. Embedded in
the fibrous stroma are branches of the hepatic artery and portal vein. Hema-
toxylin and eosin stain. × 45.

FIG. 4. Case 1. Higher-power illustration of the relatively nondilated but highly
irregular and distorted bile ducts about the periphery of one of the dilated
cystic lesions. The number of ducts is greater than is normal. Small branches
of the hepatic artery and vein lie between the ducts in the center. Foci of
hematopoiesis are evident among the cords of parenchymal epithelial cells.
Hematoxylin and eosin stain. × 190.

3

4
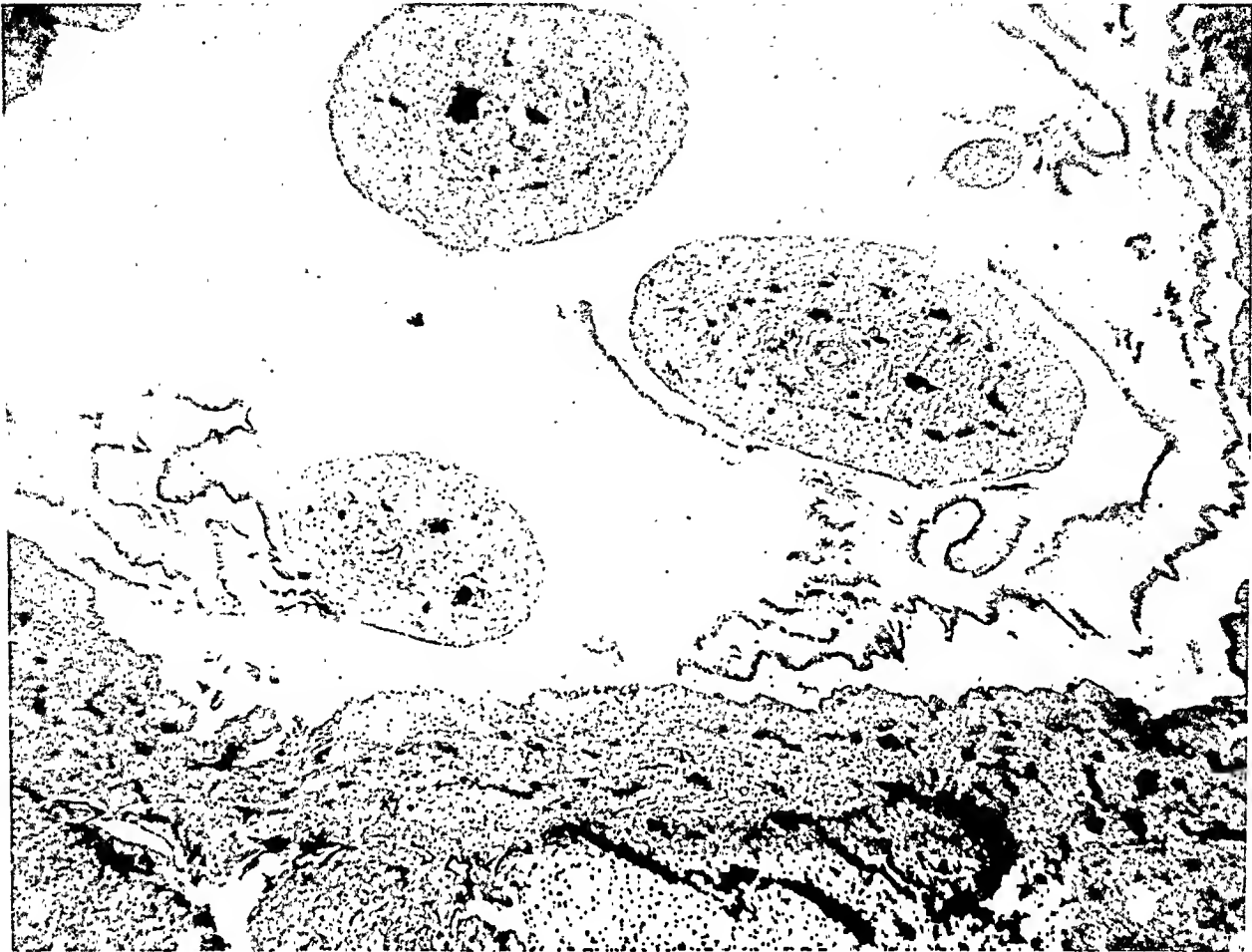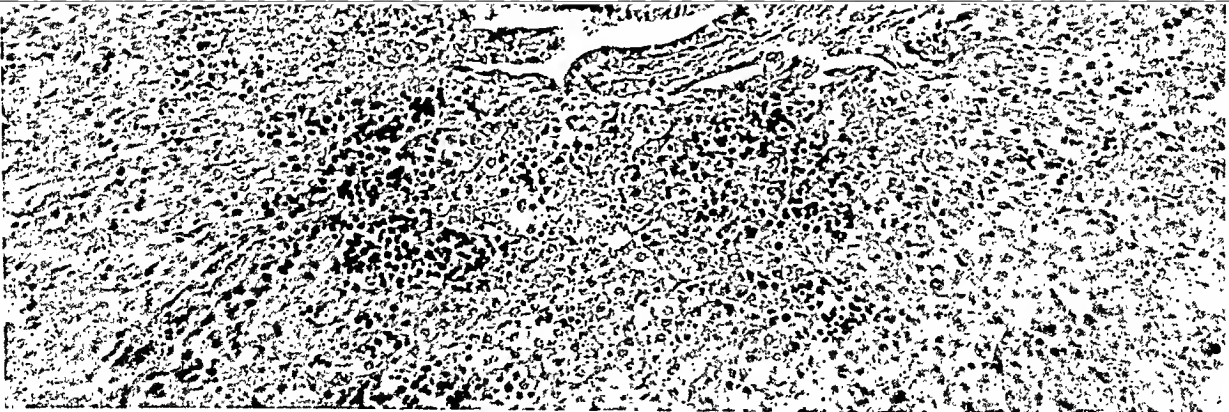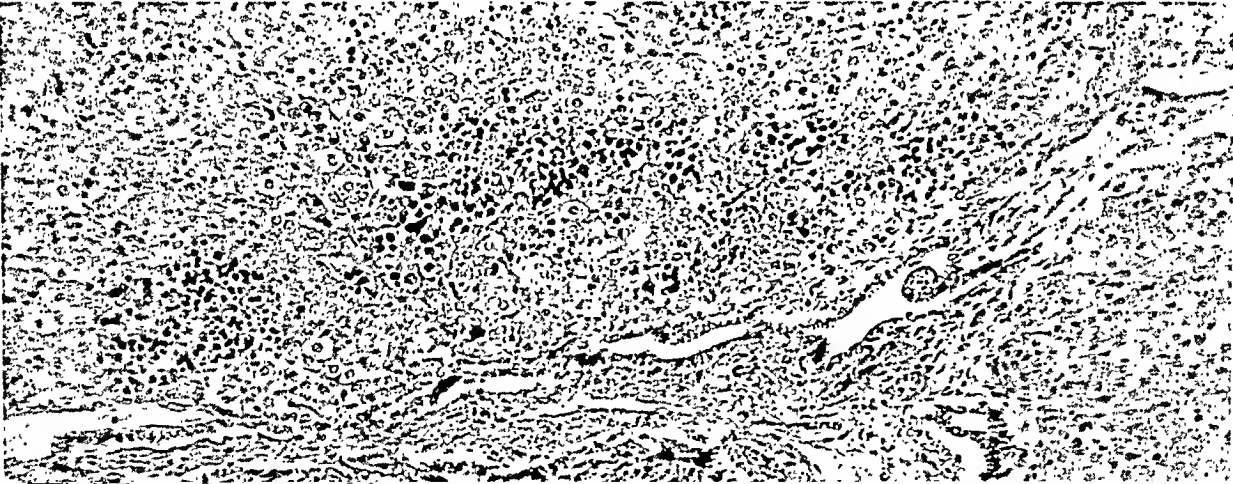
FIG. 5. Case 2. Low-power photomicrograph showing a periportal space in the center and the relatively normal lobular architecture. The centrally located circular space is a branch of the portal vein. Branches of the hepatic artery are adjacent. The vessels are completely encircled by small, irregular bile ducts which appear more numerous than is normal. Hematoxylin and eosin stain. × 23.

FIG. 6. Case 2. Higher-power illustration of a periportal space. The marked distortion and irregularity without significant dilatation of the small bile ducts are evident. There are also slightly distorted branches of the portal vein. Of note is the similarity of these ducts to those in Figure 4. Hematoxylin and eosin stain. × 190.
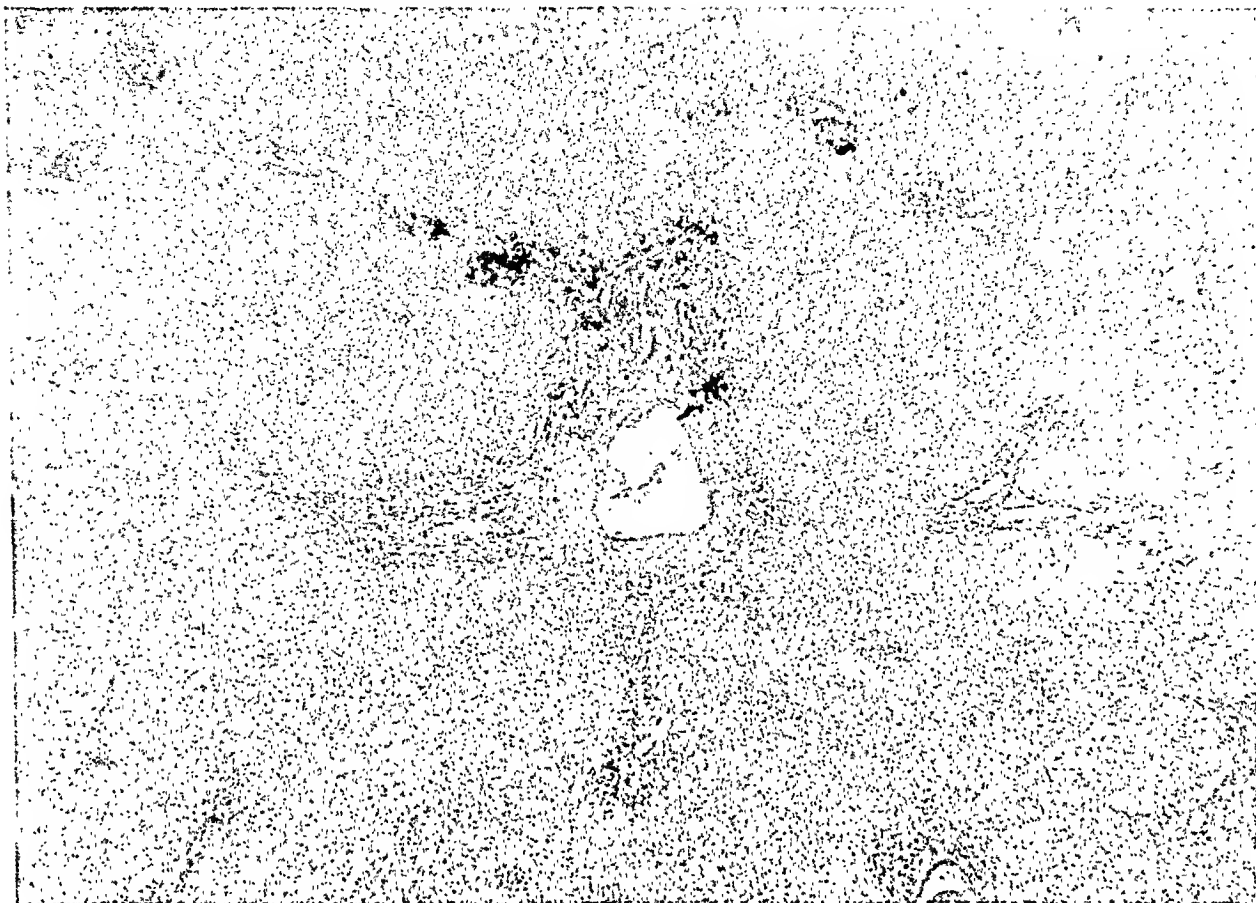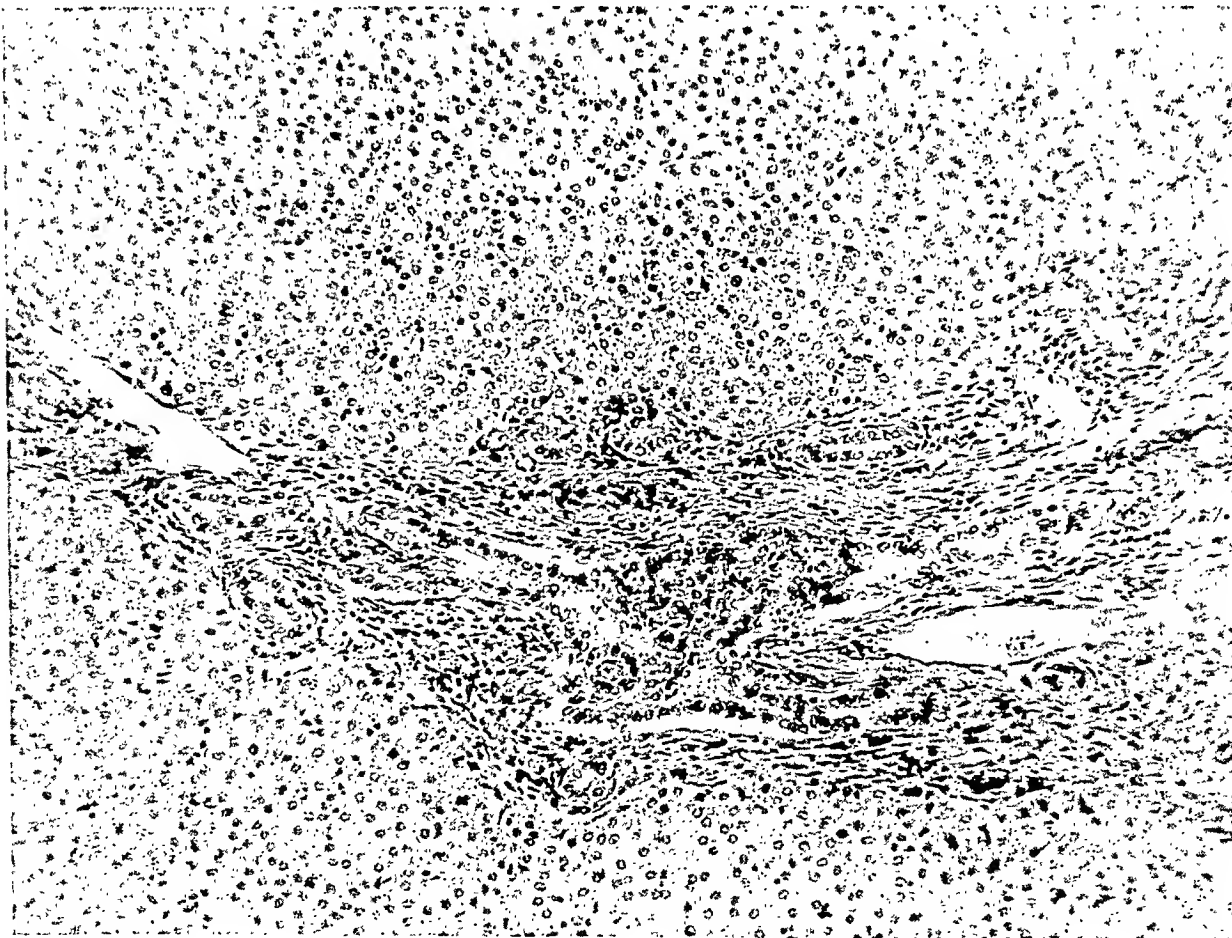
A HISTOLOGIC STUDY OF THE REACTION IN THE HAMSTER SPLEEN PRODUCED BY THE VIRUS OF COLORADO TICK FEVER [*]

William C. Black, M.D., Lloyd Florio, M.D., and Mabel O. Stewart

(From the Departments of Pathology, and Public Health and Laboratory Diagnosis, University of Colorado School of Medicine, Denver, Colo.)

In a recent study [1] it was found that the blood serum from human cases of Colorado tick fever is infectious for the golden hamster. The present report is an outgrowth of that work and constitutes a study of the histologic reaction in the hamster spleen.

Methods of Study

Twenty-four normal hamsters were sacrificed and the various tissues were removed within 30 minutes of death and fixed for 5 hours in Zenker's fluid without the addition of acetic acid. Paraffin sections were stained with hematoxylin and eosin.

Sera from 6 typical human cases (all Caucasians) of Colorado tick fever were used to infect the initial groups of hamsters. Cases 11, 12, 13, and 14 are natural instances of the disease (see Table I for histories, physical and hematologic findings). Cases 34 and 39 resulted from experimental infections and have been previously reported.[1]

Four days after the 6 initial hamster groups were injected intraperitoneally with 0.5 cc. of serum from each of the 6 human cases, the animals were anesthetized and bled as previously described.[2] A small portion of the blood was oxalated for a study of the white blood cells and the remainder allowed to clot. The pooled serum from some of the hamsters was used to inoculate new groups of animals according to the sequence of transfers shown in Text-Figure 1.

The inoculated animals were killed on the fourth day, autopsied within 30 minutes of death, and the tissues prepared in the same manner as those from the normal animals.

Since no constant nor significant lesions were found in organs other than the spleen in a comparison of the central nervous system, bone marrow, spleen, liver, kidneys, gonads, heart and lungs of the normal animals and of those of the first experimental groups, this study was limited to the spleen.

Description of Normal Hamster Spleen

The normal hamster spleen is somewhat variable in size in animals of equal age and weight. It has a thin fibrous capsule and trabeculae, with the white pulp distributed along the arterioles. The red pulp

TABLE I

History, Physical and Hematologic Findings in Natural Cases of Colorado Tick Fever Used to Inoculate Hamsters

Basophils (per cent)	0 0 1 0 0 0 0 0	1 1
Eosinophils (per cent)	0 0 1 0 1 1 0 0	0 0
Monocytes (per cent)	9 8 7 5 4 3 5 11	0 6
Lymphocytes (per cent)	63 29 53 29 63 54 45 39	54 67
Segmented forms (per cent)	17 46 22 37 17 32 42 21	16 9
Band forms (per cent)	11 17 16 29 15 10 8 29	20 17
Leukocytes in thousands per cmm.	2.25 3.55 2.80 1.80 2.00 4.05 4.35 1.50	3.40 3.85
Days after onset of symptoms	6 1 3 5 7 10 4 5	6 7
Characteristic fever	++	++
Second attack (days)	2 2	1 2
Remission (days)	2 2	1 1
First attack (days)	2 2	2 2
Photophobia	++	+
Deep ocular pain	+	
Anorexia		+
Muscle and joint pain	++	+
Backache	++	++
Headache	++	++
Chilly sensations	++	+
Incubation (days)	4 6	3 5
Tick bite	++	++
Sex	M M	M M
Age	7 23	65 12
Case number	11 12	13 14

consists of sinusoids and pulp spaces filled with blood, with groups of lymphoid cells scattered through it. In some animals these cells are present in large numbers and in a more or less diffuse arrangement. The white pulp consists of lymph follicles in which germinal centers are not well defined but with scattered large mononuclear cells with abundant pale cytoplasm interspersed with the smaller darkly staining lymphoid cells. At the periphery of the follicle there is a sharply defined, circular margin beyond which there may be several layers of somewhat larger and less darkly staining lymphoid cells (Fig. 1). The center of the follicle may normally contain deposits of brown granular pigment within mononuclear cells, and an occasional giant cell is found in the same region.

To determine whether normal serum had any effect on the spleen, 8 hamsters were each injected intraperitoneally with 0.5 cc. of normal human serum and 9 animals were injected in the same manner with normal hamster serum. Four days later the animals were sacrificed. Histologic study of the spleens revealed no change.
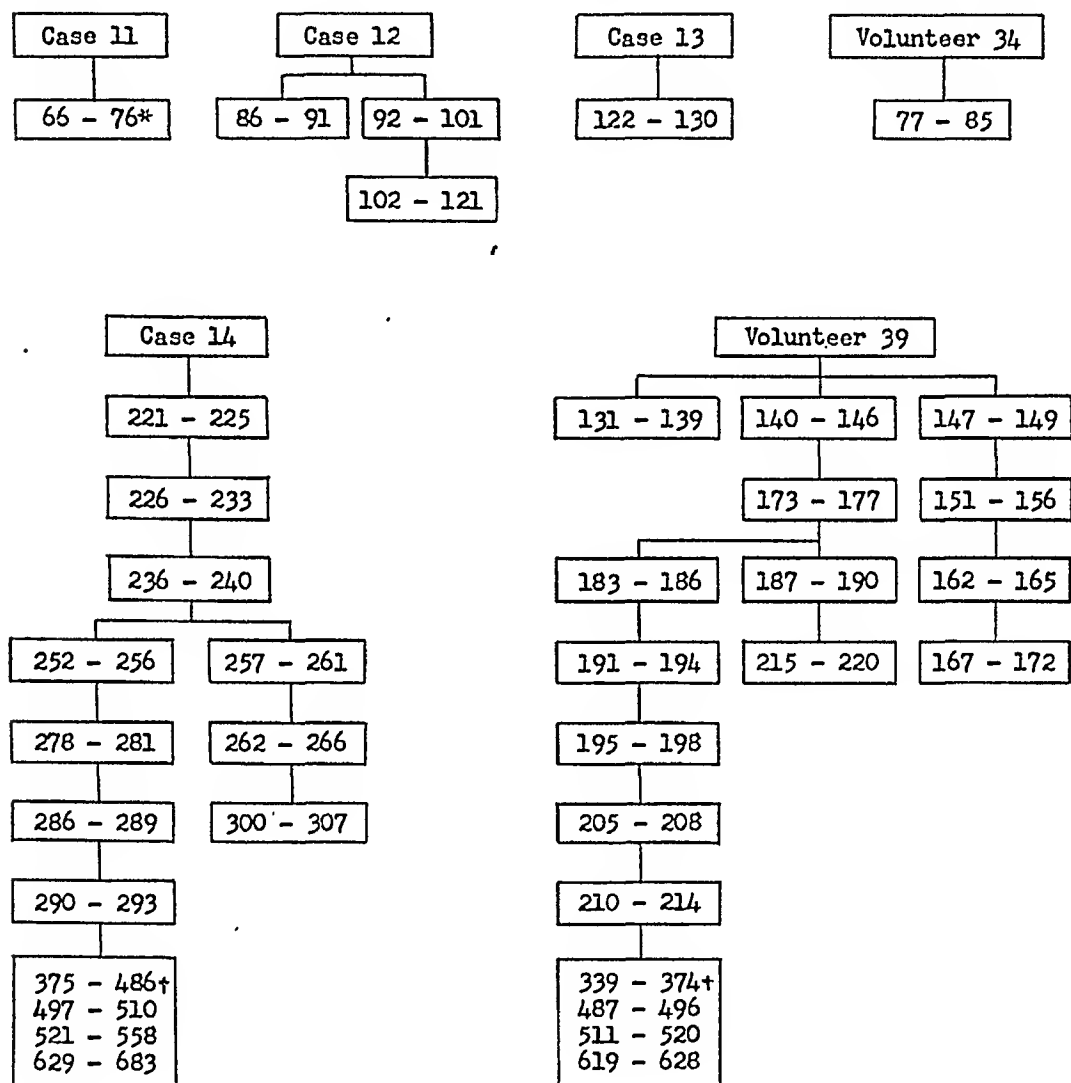
DESCRIPTION OF INFECTED HAMSTER SPLEEN

In the majority of spleens from infected animals, altera-

tions in the cellular type and arrangement of the follicular lymphoid tissue were found to be present, with variations in the extent but not in the type of reaction. There was an apparent reduction in the number of lymphoid cells in the central portion of the follicle, with the appearance

TEXT-FIGURE I

Sequence of Transfers from Human Cases of Colorado Tick Fever Through Hamsters

Case 11	Case 12		Case 13	Volunteer 34
66 – 76*	86 – 91	92 – 101	122 – 130	77 – 85
		102 – 121		

Case 14		Volunteer 39		
221 – 225		131 – 139	140 – 146	147 – 149
226 – 233			173 – 177	151 – 156
236 – 240		183 – 186	187 – 190	162 – 165
252 – 256	257 – 261	191 – 194	215 – 220	167 – 172
278 – 281	262 – 266	195 – 198		
286 – 289	300 – 307	205 – 208		
290 – 293		210 – 214		
375 – 486† 497 – 510 521 – 558 629 – 683		339 – 374† 487 – 496 511 – 520 619 – 628		

* All numbers are inclusive.

† This block of numbers represents the animals used in determining the etiology of the disease.

throughout the follicle of large pale-staining mononuclear cells mingled with polymorphonuclear leukocytes and erythrocytes. The large mononuclear cells contained small, irregularly shaped masses of dark blue-staining material resembling fragmented nuclei. The periphery of the follicle showed a partial or complete disappearance of the normal well defined margin, which was replaced by a ragged border of mononuclear

cells,. with occasional polymorphonuclear leukocytes and erythrocytes (Fig. 2). This reaction, as a whole, was graded ±, +, ++, or +++, according to its severity.

Following the usual custom in describing splenic phagocytes, the large mononuclear cells will be referred to as macrophages. Phagocytosis by the macrophages of whole lymphoid cells or of erythrocytes was not frequent. Congestion of the red pulp was not observed.

Staining methods employed in addition to hematoxylin and eosin included those of Giemsa and Gram, and Foot's modification of the Bielschowsky reticulum stain. With the Giemsa stain both eosinophilic and basophilic intracytoplasmic inclusions were seen in the macrophages. A large part of the included material was Gram-positive and evidently was composed of fragmented nuclei, presumably derived from phagocytosed lymphoid cells. Reticulum fibrils underwent some distortion but were not destroyed.

The possibility that these changes in the spleen were not caused by the virus of Colorado tick fever but were the result of some extraneous agent picked up in the serial passage of presumably normal hamster serum was eliminated in the following way. Serum from 5 healthy animals was pooled and 0.5 cc. was inoculated into each of 10 hamsters. They were bled on the fourth day and the procedure was repeated serially through 10 groups of approximately 10 animals each.[3] Sections from the spleen of each of the animals were studied. All were normal, as were the white blood cell counts.

EXPERIMENTAL RESULTS

The data on white blood cell counts, presence of bodies in the lymphocytes as described in previous papers,[1,3] and the splenic reactions in the hamster groups used, whether or not they were included in the serial transfers, are shown in Table II.

In order to determine the duration and period of maximum intensity of the splenic reaction, two experiments were carried out. In the first, one group of hamsters was inoculated with serum from volunteer 39 and another group with hamster serum after having been passed serially through 6 groups of animals from a natural instance of the disease. At least one hamster was killed daily for 8 days in each of the 2 groups. In the second experiment, the same two strains were used. The first strain had been serially passaged through 18 and the second through 20 hamster groups. Eight to 10 animals were killed daily for 5 days.

The splenic reaction was definite on the second day, most pronounced on the third, and progressively less intense on the fourth and fifth

days. The average white blood cell count was lowest on the fourth
day, at which time cytoplasmic bodies in lymphocytes also were most

TABLE II

Splenic Reactions, Mean White Blood Cell Counts, and the Presence of Cytoplasmic Bodies in Lymphocytes in Hamster Groups Inoculated with the Virus of Colorado Tick Fever

Animal numbers*	Source of material	Mean leukocytes in thousands per emm.	Number of animals with cytoplasmic bodies in lymphocytes (100 cell hemogram)	Splenic reactions					
				0	±	+	++	+++	Not done
66– 76	Case 11	6.72 ± 2.22	0	6	3	1	0	1	
77– 85	Volunteer 34	5.37 ± 2.33	0	6	1	1	1	0	
86– 91	Case 12	6.39	0	6	0	0	0	0	
92–101	Case 12	5.03	0	10	0	0	0	0	
102–121	92–101	6.16	0	20	0	0	0	0	
86–121		5.89 ± 1.85							
122–130	Case 13	7.63 ± 0.79	4	0	2	4	2	1	
131–139	Volunteer 39	6.07	3	0	3	1	2	3	
140–146	Volunteer 39	4.16	3	1	2	1	1	2	
147–150	Volunteer 39	4.53	3						4
151–156	147–149	4.77	4	0	3	1	2	0	
157–166	151–156	6.01	4	0	1	4	1	4	
167–172	162–165	6.00	0	5	0	1	0	0	
173–182	140–146	4.49	3	0	5	4	1	0	
183–186	173–177	2.54	0	0	1	1	2	0	
187–190	173–177	5.86	0	1	1	2	0	0	
191–194	183–186	3.33	1	1	0	2	1	0	
195–204	191–194	3.80	1	0	0	5	2	3	
205–208	195–198	5.79	3	0	1	2	1	0	
209–214	205–208	4.86	3	3	1	1	1	0	
215–220	187–190	4.90	3	2	1	1	1	1	
131–220		4.87 ± 1.91							
221–225	Case 14	4.66	4	3	2	0	0	0	
226–235	221–225	5.01	5	1	4	1	3	0	1
236–251	226–233	5.84	2	4	5	4	2	0	1
252–256	236–240	5.36	3	0	1	3	1	0	
257–261	236–240	4.49	0	0	5	0	0	0	
262–277	257–261	3.61	0	2	2	5	4	3	
278–285	252–256	4.62	2	0	4	2	2	0	
286–289	278–281	4.04	0	0	0	2	2	0	
290–299	286–289	4.18	1	1	0	3	3	3	
300–307	262–266	4.24	3	0	3	3	1	1	
221–307		4.63 ± 1.86							
†131–307		4.75 ± 1.90							
‡339–486	...	4.77 ± 2.08	69	16	10	26	34	62	
487–510		5.43 ± 2.96	13	4	4	8	4	4	
511–558		5.34 ± 2.99	17	2	2	4	8	31	1
619–683		4.30 ± 1.73	16	14	4	12	17	18	
‡280 infected animals		4.81 ± 2.30							
§522 infected animals		4.76 ± 2.11							

* All numbers are inclusive.
† Since the difference between the means in each of the two major strains used is less than 1 standard deviation, the data for the two groups were combined.
‡ Animals 339–683 were used to determine the etiologic factor.
§ Since the difference between the means of animals 131–307 and those used in determining the etiologic factor is less than 2 standard deviations, they were combined.

commonly found. The splenic reactions are correlated with the total white blood cell counts and the presence of cytoplasmic bodies in Table III.

After the sixth or seventh day, the spleens of recovered animals were indistinguishable histologically from those of normal hamsters.

TABLE III

Duration of Splenic Reaction Correlated with the Mean White Blood Cell Count and Cytoplasmic Bodies in Lymphocytes

Days after inoculation	Animal numbers*	Mean leukocytes in thousands per cmm.	Number of animals with cytoplasmic bodies in lymphocytes (100 cell hemogram)	Splenic reactions				
				0	±	+	++	+++
1	308–310†	6.10	0	2	1	0	0	0
	684–691‡	6.99	0	8	0	0	0	0
	Both groups	6.75	0	10	1	0	0	0
2	692–699	6.27	0	0	0	1	6	1
	311–314	6.42	1	1	0	2	1	0
	Both groups	6.32	1	1	0	3	7	1
3	315–318	3.64	1	0	0	1	3	0
	700–709	4.84	1	0	0	0	1	9
	Both groups	4.49	2	0	0	1	4	9
4	710–719	3.84	4	0	0	4	3	3
	319–321	3.82	2	1	1	0	1	0
	Both groups	3.84	6	1	1	4	4	3
5	322–325	7.65	1	3	1	0	0	0
	720–727	5.29	0	3	0	4	1	0
	Both groups	6.15	1	6	1	4	1	0
6	326–329	4.43	0	4	0	0	0	0
7	330–333	10.15	0	4	0	0	0	0
8	334–336	4.16	0	2	0	1	0	0
9	337	6.60	0	1	0	0	0	0
10	338	7.50	0	1	0	0	0	0

* All numbers are inclusive.
† Numbers 308–338 represent the first experimental group.
‡ Numbers 684–727 represent the second experimental group.

DISCUSSION

No direct analogy can be found for this splenic reaction. Although gross swelling is not a prominent feature, the term acute splenic tumor may be applied to it. In man, many acute infectious diseases produce swelling of the spleen, with cellular reactions classified as either the red or gray type of acute splenic tumor. Comparison of the reaction in the hamster spleen in Colorado tick fever with the human spleen in various infections shows practically no resemblance to the gray type, and only a very general similarity to the red type.

Experimental tularemia in the hamster does not give a splenic picture such as we have observed.[4] It is impossible to say that the splenic reaction in the hamster with Colorado tick fever is pathognomonic of this disease. This decision must await further work on other diseases with these animals.

It will be seen that animals inoculated with serum from case 12 failed to show any deviation from normal in the histologic picture of the spleen. This is the only instance in which the hamster groups did not develop a characteristic splenic reaction as a result of inoculating serum from an individual who was considered to have a typical case of Colorado tick fever.

With the exception of the 36 animals inoculated with serum from case 12, only 72 of the 484 spleens studied failed to show a positive reaction in the inoculated animals. Sixteen animals (3 per cent) failed to show a white blood cell count under 6,000 cells per cmm., cytoplasmic bodies in the lymphocytes, or a positive splenic reaction. The splenic reaction is most likely to be negative in the original human-to-hamster transfer.

It must be pointed out that the results recorded in Table III constitute two separate experiments that possibly are not comparable. In the second experiment, the infectious agent had been passaged through many more hamster groups than in the earlier experiment.

SUMMARY

1. A study of the spleens from hamsters infected with Colorado tick fever showed alterations in the cellular type and arrangement of the follicular lymphoid tissue as well as a partial or complete disappearance of the normal well defined follicular margin.

2. This reaction was observed in 79.3 per cent of 520 infected animals. These figures include the one instance of the disease that failed to show this reaction in a series of 36 animals.

3. Normal hamster serum passaged through 10 groups of animals failed to elicit these responses.

4. The splenic reaction was observed first on the second day following inoculation, reached its height on the third day, and continued through the fifth day.

REFERENCES

1. Florio, L., Stewart, M. O., and Mugrage, E. R. The experimental transmission of Colorado tick fever. *J. Exper. Med.,* 1944, 80, 165–188.

2. Stewart, M. O., Florio, L., and Mugrage, E. R. Hematological findings in the golden hamster (*Cricetus auratus*). *J. Exper. Med.*, 1944, 80, 189–196.

3. Florio, L., Stewart, M. O., and Mugrage, E. R. The etiology of Colorado tick fever. *J. Exper. Med.*, 1946, 83, 1–10.

4. Lillie, R. D., and Larson, C. L. Pathology of experimental tularemia in the golden hamster (*Cricetus auratus*). *U. S. Pub. Health Rep.*, 1945, 60, 1243–1253.

DESCRIPTION OF PLATE

PLATE 39

FIG. 1. Normal hamster spleen. × 140.

FIG. 2. Positive reaction (++) in hamster spleen. A central arteriole may be seen to the left of the center of the field. × 140.
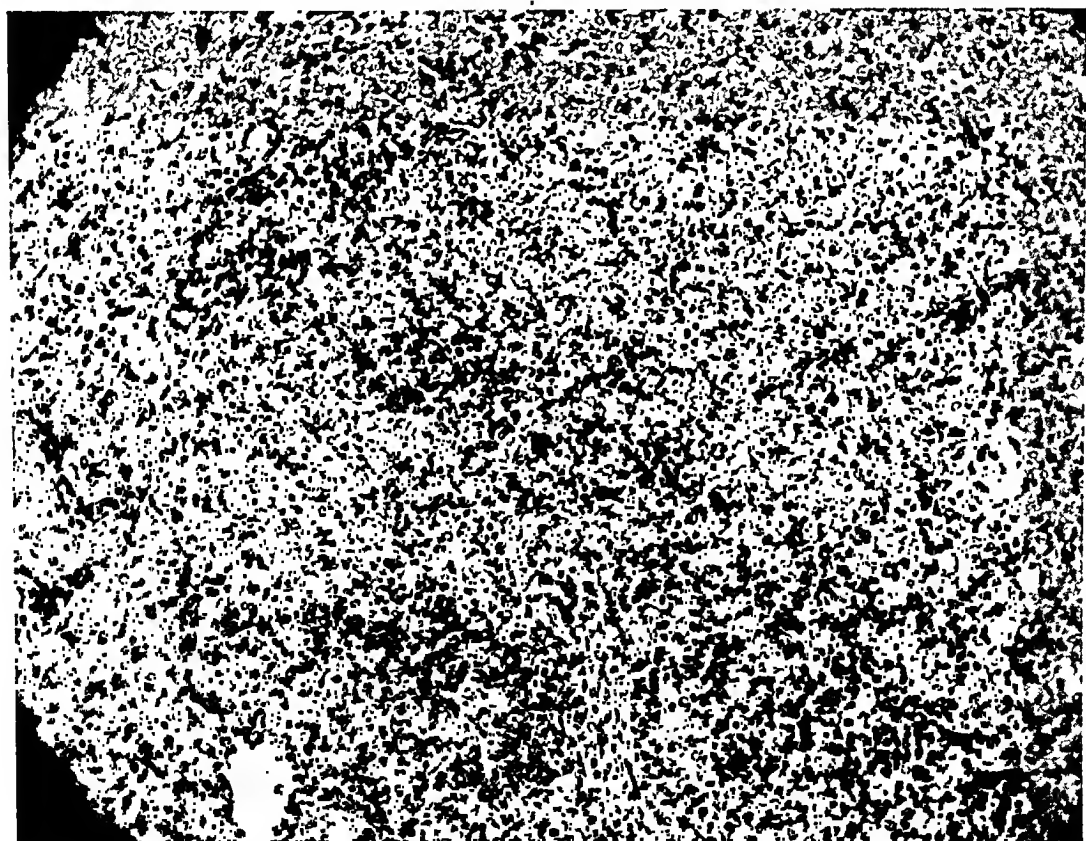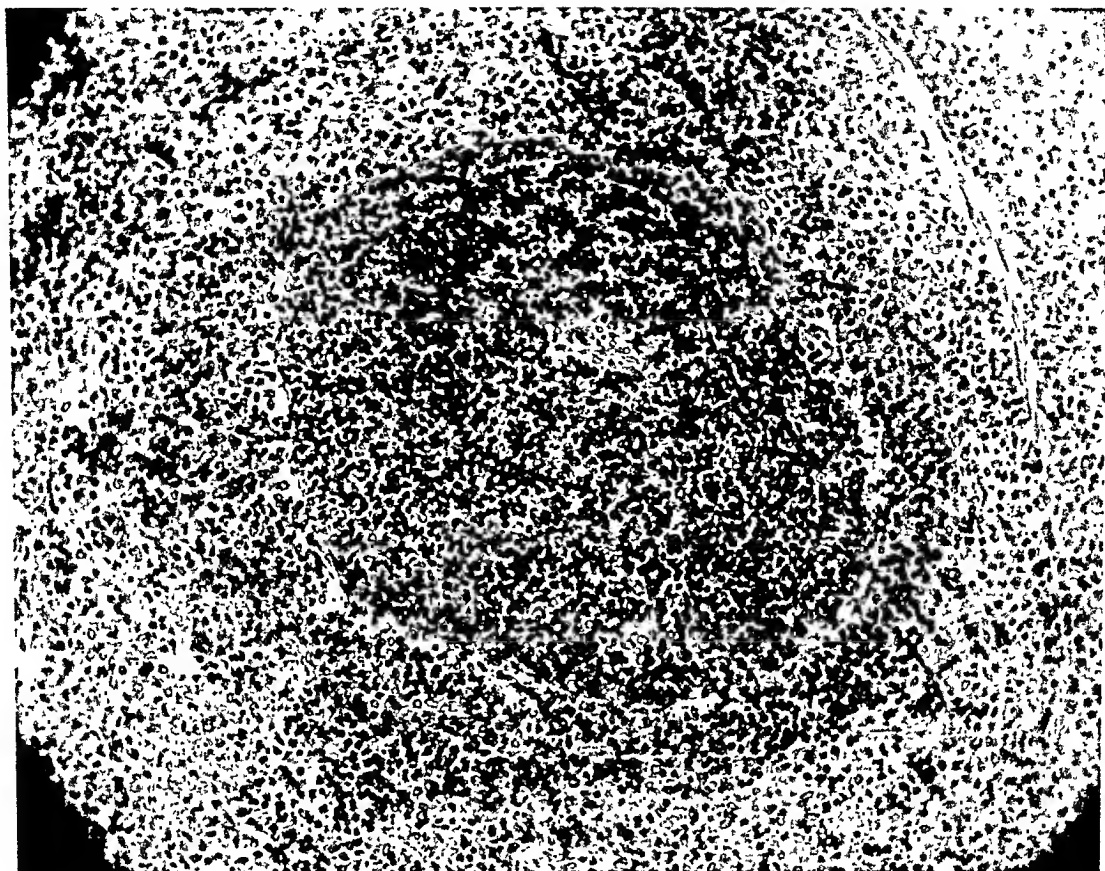
BLINDNESS IN DUCKS ACCOMPANYING HYPOGLYCEMIA

A Clinical and Pathological Study *

R. H. Rigdon, M.D., and D. E. Fletcher, Ph.D.

(*From the Department of Pathology, University of Arkansas, School of Medicine, Little Rock, Ark.*)

In a recent study of hypoglycemia in ducks,[1] it was observed that birds with a marked reduction of blood glucose wandered purposelessly about the laboratory and frequently ran into objects. Furthermore, they did not react when a hand was moved near their eyes. A disturbance in vision also has been observed in ducks with a severe malarial infection. The latter birds had acute degenerative lesions in the optic nerve and brain tissue.[2] Clinical observations, the glucose levels of the blood, and the pathological lesions as observed in the central nervous system of ducks given insulin are reported in this paper.

Materials and Methods

The ducks were white Pekins, 4 to 6 weeks of age. They were given 30 units per kg. of both plain insulin, "Iletin," and protamine zinc insulin. The former preparation was given intravenously and the latter subcutaneously. Approximately 8 hours following the first injection of insulin, a second injection of 15 units of protamine zinc insulin per kg. of body weight was given. A third dose of protamine zinc insulin, 30 units per kg., was given approximately 16 hours following the second injection.

Blood for glucose determination was obtained from either the inner surface of the wing or the web of the foot. The determinations were made according to the Hagedorn-Jensen method as given by Peters and Van Slyke,[3] except that the blank was added to, instead of subtracting from, the titration values.

The birds used for pathological study were sacrificed by decapitation at intervals varying from 2 to 60 days. The brain and the eyes were removed from each bird while the spinal cord and ganglia were obtained from some of them. One-half of each tissue was put into Bouin's solution and the remainder into a 10 per cent solution of formalin. Paraffin sections were prepared from the Bouin-fixed tissues and they were stained with hematoxylin and eosin, thionin, Weil's myelin sheath stain, and ammonical silver hydroxide.

Some of the ducks were given a 5.0 per cent solution of glucose intravenously and were fed by stomach tube following the development of neurological manifestations. This was done to obtain material for study of the pathological changes in the nervous system at varying intervals after recovery from the acute phase of hypoglycemia.

EXPERIMENTAL FINDINGS

Thirteen ducks were given insulin in this experiment. A few of these birds were less active than normally 8 hours following the first injection. Blindness first appeared on the second experimental day, approximately 6 hours after the third injection of insulin. Each bird in this group was either partially or completely blind at this time. They were weak and usually sat together in a corner of the battery. They neither ate nor drank during this period. Usually the birds had convulsions on the third day; however, duck 1441 had a convulsion on the second day. Five ducks (1436, 1441, 1451, 1453, and 1454) were blind, or had convulsions, and were killed between the second and fourth day of the experiment.

Four of the ducks (1440, 1450, 1455, and 1456) were given glucose intravenously and food and water by stomach tube after they became blind (Table I). Duck 1455 had numerous convulsions on the 5th day, became moribund, and was killed. The other three ducks in this group recovered their sight by the 5th day and appeared normal at the time they were killed on the 17th, the 25th, and the 39th day of the experiment.

The glucose level of the blood was followed in three ducks (1436, 1440, and 1441) as shown in Table I. The normal level of blood sugar in these three birds was 188, 159, and 172 mg. per cent, respectively. The blood glucose was approximately 100 mg. per cent on the afternoon of the first day. On the afternoon of the second day, it was 57 and 74 mg. per cent in two birds, which were blind. One duck at this time had convulsions and died. On the third day the blood sugar varied between 50 and 66 mg. per cent. One of these ducks (1436) was killed on the third day. The third duck in this group (1440) was given glucose, food, and water on the third day of the experiment. The blood sugar was 197 mg. per cent on the morning of the fourth day. Vision had improved by this time and apparently it was normal on the fifth day following the first injection of insulin. This duck was killed on the 39th experimental day.

Four ducks (1539, 1540, 1541, and 1542) were given three injections of insulin at the same times as those given to the other ducks used in this study. Each bird was blind on the third day of the experiment.

During this time, duck 1539 had several convulsions and was killed on the third day. Duck 1542 was weak and had many convulsions during the first 4 days of the experiment. It was given glucose intravenously and food and water by stomach tube. Ducks 1540 and 1541 were blind on the third day; however, their sight returned by the fourth day.

The glucose level of the blood was followed in these 4 ducks. It was 68 mg. per cent in duck 1539 on the day it was having convulsions and was killed. The blood glucose in duck 1542 was only 84 and 77 mg. per cent on the third day, 57 and 56 mg. per cent on the fourth day, and 41 mg. per cent on the fifth day. By the eighth experimental day the glucose level was normal. This bird had many convulsions during this time and was given glucose intravenously. Sight was markedly impaired until the eighth day. The lowest glucose level was 90 mg. per cent in ducks 1540 and 1541. Sight returned in these two birds by the fourth day.

Ducks 1540, 1541, and 1542 were kept for 60 days. During this time they showed some incoordination of movement. One could not be sure that sight was impaired; however, at times their actions suggested some disturbance in vision.

There were no macroscopical lesions observed at autopsy in either the birds sacrificed during the early phase of hypoglycemia or in those killed 60 days later. Microscopically, however, the small blood vessels were dilated and filled with blood in all sections removed from the brains and spinal cords of ducks sacrificed during the first 5 days of the experiment. The endothelial cells were swollen and the perivascular spaces were enlarged. The tissue was loose and in areas it appeared reticulated. The nerve fibers were swollen. The retina was edematous. Many focal areas of degeneration of various sizes and shapes were present in the substantia alba and in the great fiber tracts throughout the diencephalon, brain stem, and cerebellum. These lesions were present in the myelinated fiber tracts and in the reticular formation. They were numerous in the optic tracts and in the optic nerves (Figs. 1 and 2). Many of the nerve cells in the brain and spinal cord were swollen and showed central chromatolysis. This change was especially pronounced in the cells of the brain stem and cerebellum.

The nerve fibers in the focal areas of degeneration were swollen and demyelinated, as shown in the myelin sheath preparations. The axis cylinders were refractory to silver impregnation and many were fragmented. The nerve and glial cells remaining in these focal areas were swollen, pale-staining, and frequently fragmented. The nerve cells and fibers in the peripheral nerves and ganglia showed only minimal changes.

TABLE I

Effect of Hyperinsulinism on Ducks

Exper. day	1441	1451	1436	1453	1454	1455	1450	1456	1440
1	Weight, 1370 gm.; 9 a.m., b.s.,* 172; 9:30 a.m., insulin;† 1 p.m., b.s., 99; 4:30 p.m., insulin	Weight, 1320 gm.; 8:30 a.m., insulin; 4:30 p.m., insulin	Weight, 1480 gm.; 9 a.m., b.s., 188; 9:30 a.m., insulin; 4:30 p.m., insulin	Weight, 1425 gm.; 8:30 a.m., insulin; 4:30 p.m., insulin	8:30 a.m., insulin; 4:30 p.m., insulin	Weight, 1555 gm.; 8:30 a.m., insulin; 4:30 p.m., insulin	Weight, 1475 gm.; 8:30 a.m., insulin; 4:30 p.m., insulin	Weight, 1075 gm.; 8:30 a.m., insulin; 4:30 p.m., insulin	Weight, 1480 gm.; 9 a.m., b.s., 159; 9:30 a.m., insulin; 1 p.m., b.s., 95; 4:30 p.m., insulin
2	9 a.m., b.s., 108; 9 a.m., insulin; 2 p.m., convulsion, died	8:30 a.m., insulin; 3:30 p.m., blind; 3:15 p.m., killed	9 a.m., b.s., 124; 9 a.m., insulin; 2:30 p.m., blind; 4 p.m., b.s., 74	8:30 a.m., insulin; 4 p.m., impaired vision	8:30 a.m., insulin; 3:00 p.m., blind	8:30 a.m., insulin; 3 p.m., impaired vision	8:30 a.m., insulin; 3 p.m., blind	8:30 a.m., insulin; 3:00 p.m., blind	9 a.m., b.s., 146; 9 a.m., insulin; 2:30 p.m., blind; 4:30 p.m., b.s., 57
3			9 a.m., b.s., 59; 2 p.m., blind; 2:15 p.m., killed	10 a.m., blind, convulsion; 11 a.m., convulsion; 4 p.m., convulsion; 4:15 p.m., killed	8:30 a.m., blind; 6:00 p.m., blind	9 a.m., blind; 4 p.m., blind; convulsion, glucose‡	9 a.m., blind; 4 p.m., convulsion, glucose; 8 p.m., sight improving	8:30 a.m., blind; 3 p.m., impaired vision, glucose; 8 p.m., sight improving	9 a.m., b.s., 50; 11 a.m., glucose; 2:30 p.m., glucose; 4 p.m., b.s., 66; 4:30 p.m., glucose

4	8:30 a.m., blind; 2 p.m., blind; 2 p.m., killed	8:30 a.m., glucose, blind; 2 p.m., blind; 3 p.m., glucose; 8 p.m., blind	8 a.m., blind, glucose; 2 p.m., blind; 3 p.m., glucose	8 a.m., glucose; 9 a.m., normal; 2 p.m., normal	9 a.m., b.s., 197; 10 a.m., glucose, impaired vision; 4 p.m., b.s., 274
5		9:30 a.m., convulsion, glucose; 1 p.m., blind; 9 p.m., convulsion; 11 p.m., convulsion; 12 p.m., moribund, killed	1 p.m., impaired vision; 9 p.m., normal	Normal	Normal
			Killed on 17th day	Killed on 25th day	Killed on 39th day

* b.s. = blood sugar in mg. per cent.

† On the morning of the first day, 30 units of straight insulin were given intravenously and 30 units of protamine insulin were given subcutaneously per kg. On the afternoon of the first day, 15 units of protamine insulin were given subcutaneously per kg. On the morning of the second day, 15 units of protamine insulin were given subcutaneously per kg.

‡ The glucose is a 5.0 per cent solution, usually 10 cc. given intravenously.

Histological studies of the nervous tissue from ducks sacrificed 17 days or longer following the injection of insulin showed small focal areas of degeneration in the fiber tracts similar to those observed in the acute phase except that they were "filled in" by nerve fibers and glial elements (Figs. 3 and 4). The nerve fibers were normal in size and the tissue was more compact than that observed during the acute phase of hypoglycemia. Only minimal alterations were present in the nerve cells at this time. No significant changes were observed in either the peripheral nerves or the ganglia.

DISCUSSION

The ducks were blind on the second day following the injection of large amounts of insulin. At this time the glucose levels of the blood were markedly decreased. This disturbance in vision was only temporary since sight gradually returned following the return of the blood glucose to normal levels. Edema with separation of the nerve cells and fibers, and focal necrosis characterized the early changes in the brain, optic nerve, and retina of these ducks. After recovery from the hypoglycemia, these lesions markedly regressed, as indicated by the subsequent histological studies. A disturbance in coordination and vision accompanying hyperinsulinism has been observed in the pigeon [4] and the rabbit.[5]

The pathological changes observed in the brains of these ducks with hypoglycemia are similar to the lesions observed in ducks with a severe malarial infection.[2] It has been suggested elsewhere [6,7] that the pathological lesions occurring in the brains of ducks with malaria result from anoxia. In cerebral glycolysis both glucose and oxygen are essential. It appears, therefore, from the above experimental studies that a similar lesion may develop in the brains of ducks if either anoxia or hypoglycemia occurs. Himwich [8] has recently reviewed the phenomenon of cerebral glycolysis and has emphasized the fact that different parts of the brain glycolyze at different rates. It is suggested from this study that the optic nerve in the duck may be very susceptible to a lack of glucose.

The return of sight following the return of the glucose levels of the blood to normal indicates that the pathological change is reversible. The early lesions in the brain associated with hypoglycemia suggest that cellular degeneration is minimal while edema is marked. Therefore, with the elapse of time and the restoration of the metabolic processes to normal, the pathological processes regress. These lesions may not heal completely, as shown by the histological studies made on birds that survived the acute phase of glycemia. Residual damage has been observed in the brains of ducks following the acute manifestations

of malaria.[2] Kabat, Dennis, and Baker [9] also observed residual damage in dogs previously kept under anoxic conditions. It may be that the number of areas of degeneration are too few and their location in the brains of hypoglycemic ducks are such that clinical changes are not pronounced. It is more difficult, of course, to discern either a few or minimal neurological changes in the duck than in man. Although carbohydrate metabolism in the duck differs from that in man, it is of interest to know that pathological changes have been reported in human brains following the injection of large amounts of insulin.[10,11]

The sameness of the pathological lesions in the brains of hypoglycemic ducks and those kept under anoxic conditions is interesting when considered along with the study of Gellhorn, Ingraham, and Moldavsky [12] on the influence of hypoglycemia on the sensitivity of the central nervous system to oxygen want. They wrote:

"If the theory.... is correct, that a lowering of the blood sugar reduces both the sugar and oxygen consumption of the brain, the rate of oxidation in the brain should be diminished to such an extent that a condition prevails comparable to that induced by inhalation of low oxygen tensions. If this were the case, lowering of the blood sugar during a mild degree of oxygen deficiency would induce symptoms of more severe anoxemia .... than would occur at normal blood sugar levels. This would mean, in other words, that as the blood sugar level progressively falls the increment of rise in the blood pressure from inhalation of a given oxygen tension would steadily increase. This indeed had proven to be the case."

The fact that oxidative processes in the brain are diminished in hypoglycemia appears to be a more significant factor in the production of the cerebral lesions than that they result only from the convulsions, as suggested by Grayzel.[13] Terplan [14] likewise does not believe that mechanical factors alone produce the type of cerebral lesions observed in hypoglycemia. The study of Glickman and Gellhorn [15] furthermore would suggest that the lesions observed in hypoglycemia are not the result of convulsions. These investigators pointed out the fact that low blood sugar interferes with the oxygenation of the central nervous system. Therefore, a mild degree of oxygen deficiency under conditions of low blood sugar leads to symptoms which are similar to those obtained at a normal blood sugar level only under conditions of extreme anoxia.

SUMMARY

Ducks made hypoglycemic by the injection of large amounts of insulin will lose their sight. Their sight returns, however, with a return of the blood sugar to normal levels. Focal areas of edema and degeneration occur in the brain, optic nerves, and retina of these hypoglycemic birds. These lesions are similar to those observed in the brains of anoxic ducks, and apparently they result from a disturbance in cerebral glycolysis.

REFERENCES

1. Marvin, H. N., and Rigdon, R. H. Terminal hypoglycemia in ducks with malaria. *Am. J. Hyg.*, 1945, 42, 174–178.

2. Fletcher, D. E., and Rigdon, R. H. Neurologic manifestations associated with malaria. A clinical and pathological study in ducks. *Arch. Neurol. & Psychiat.*, 1946, 55, 35–42.

3. Peters, J. P., and Van Slyke, D. D. Quantitative Clinical Chemistry. Williams & Wilkins Co., Baltimore, 1932, 2.

4. Riddle, O. Resistance of pigeons to the lethal action of iletin (insulin) with observed effects on reproduction. *Proc. Soc. Exper. Biol. & Med.*, 1922–23, 20, 244–247.

5. Banting, F. G., Best, C. H., Collip, J. B., MacLeod, J. J. R., and Noble, E. C. The effect of pancreatic extract (insulin) on normal rabbits. *Am. J. Physiol.*, 1922, 62, 162–176.

6. Rigdon, R. H. The pathological lesions in the brain in malaria. *South. M. J.*, 1944, 37, 687–694.

7. Rigdon, R. H., and Fletcher, D. E. Lesions in the brain associated with malaria. A pathological study on man and on experimental animals. *Arch. Neurol. & Psychiat.*, 1945, 53, 191–198.

8. Himwich, H. E. Energy metabolism. *Ann. Rev. Physiol.*, 1945, 7, 181–200.

9. Kabat, H., Dennis, C., and Baker, A. B. Recovery of function following arrest of the brain circulation. *Am. J. Physiol.*, 1941, 132, 737–747.

10. Terplan, K. Changes in the brain in a case of fatal insulin shock. (Abstract.) *Arch. Path.*, 1932, 14, 131–132.

11. Warren, S. The Pathology of Diabetes Mellitus. Lea & Febiger, Philadelphia, 1938, ed. 2, 246 pp.

12. Gellhorn, E., Ingraham, R. C., and Moldavsky, L. The influence of hypoglycemia on the sensitivity of the central nervous system to oxygen want. *J. Neurophysiol.*, 1938, 1, 301–312.

13. Grayzel, D. M. Changes in the central nervous system resulting from convulsions due to hyperinsulinism. *Arch. Int. Med.*, 1934, 54, 694–701.

14. Terplan, L. Histopathological changes in marked swelling of the brain. (Abstract.) *Am. J. Path.*, 1937, 13, 664–667.

15. Glickman, N., and Gellhorn, E. The effect of oxygen deficiency on the sensitivity of rats to insulin. *Am. J. Physiol.*, 1938, 121, 358–363.

DESCRIPTION OF PLATE

PLATE 40

FIGS. 1 and 2. Many focal areas of edema and degeneration of nervous tissue occur in the optic nerve and tract, optic tectum, brain stem, and cerebellum of ducks given large quantities of insulin. At this time the birds are blind and the lesions are characterized by marked edema, degeneration of the nerve and of glial cells and fibers. Figure 1 is a section from the optic tectum of duck 1436, killed on the third experimental day. Figure 2 is a section from the optic nerve of duck 1453, killed on the third experimental day. Hematoxylin and eosin stain. × 440.

FIGS. 3 and 4. The acute lesions apparently are reversible since the ducks recover their sight when the blood glucose returns to normal levels. The acute focal lesions gradually are filled in by nerve fibers and glial elements. This reparative process is not complete in all birds by the 60th day. Figure 3 is a section from the optic tectum of duck 1456, killed on the 25th experimental day. Figure 4 is a section from the optic nerve of duck 1440, killed on the 39th experimental day. Hematoxylin and eosin stain. × 440.
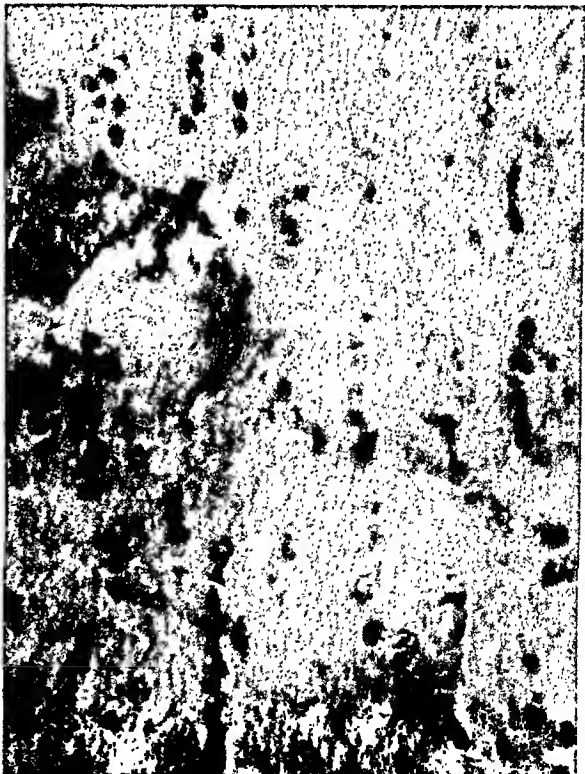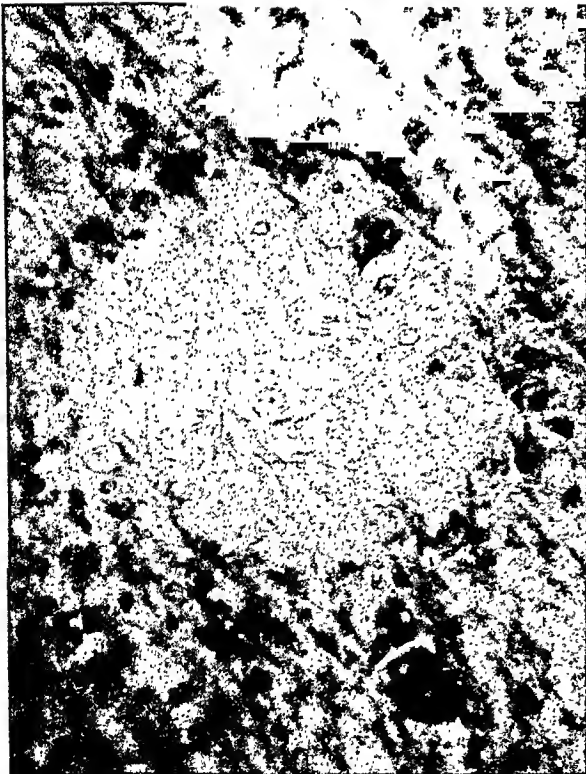
Rigdon and Fletcher

Blindness in Hypoglycemic Ducks

PLASMACYTOMA OF THE STOMACH

REPORT OF A CASE *

HOWARD SCHWANDER, M.D., JAMES ESTES, M.D., and WILLIAM G. COOPER, M.D.

(*From the Departments of Pathology and Surgery, University of Arkansas, School of Medicine, Little Rock, Ark.*)

Extramedullary plasma cell tumors are relatively uncommon. Those occurring in the gastrointestinal tract are extremely rare. Hellwig [1] recently reviewed 127 cases reported between 1905 and 1942, and added one of his own. Of these tumors, 63 arose in the upper respiratory tract, 47 in the conjunctiva, 4 in the lymph nodes, 2 each in the thyroid gland and the skin, and 1 each in the pleura, lacrymal gland, kidney, spermatic cord, vulva, and gastrointestinal tract.

No case of primary plasmacytoma of the stomach was found in a review of the literature.† Brown and Liber [2] cited the report by Vasiliu and Popa of a case with multiple ulcerated nodules in the mucosa of the stomach and intestines. These lesions were composed of plasma cells. Recently we found a plasmacytoma in a resected stomach, and the case was subsequently studied at autopsy.

REPORT OF CASE

The patient was a colored male farmer, 42 years old, whose chief complaint was "stomach trouble." He had been in good health until 7 years prior to admission. The first symptom was pain in the right lower quadrant gradually extending to the epigastrium. This was associated with frequent vomiting and inability to retain food. The pain was severe enough to require bed rest for a few weeks. Following this acute onset, the patient had slight, intermittent abdominal cramps for 6½ years. These were not severe enough to interfere with his work.

The patient experienced epigastric pain and vomited following breakfast 3 months prior to admission. This vomiting persisted, and was postprandial. Two tarry stools were observed by the patient 2 weeks prior to admission. Since his first attack he had been constipated; this was accentuated following his second attack. His average weight was 150 pounds, but at the time of admission it was only 105 pounds.

Laboratory Data. Complete pyloric obstruction was demonstrated roentgenologically. Roentgenograms of the heart, lungs, and long bones were normal. On two examinations the gastric contents were negative for free hydrochloric acid, and a large residuum was present. A Kahn test of the blood was negative. The red blood cell count was 4.4 million and the hemoglobin was 10.25 gm. The white blood cell count was 6,500, with 52 per cent polymorphonuclear leukocytes, 40 per cent lymphocytes, 4 per cent staff cells, 2 per cent juveniles, and 2 per cent eosinophils.

Clinical Course. A diagnosis of complete pyloric obstruction, due probably to a duodenal ulcer, was made, and an exploratory laparotomy was performed.

A smooth, firm annular mass, 3.0 cm. in width, was present in the wall of the stomach in the prepyloric region. It did not extend into the surrounding tissue. The regional lymph nodes varied from 1 to 2 cm. in diameter. They were soft and freely movable. No metastases were found in the viscera. A subtotal gastric resection was performed. The postoperative course was considered normal, and the patient was allowed to get out of bed on the 10th postoperative day. On the 14th day, while dressing to go home, he became weak, went to bed, and expired immediately.

Pathologic Examination of the Surgical Specimen

The surgical specimen consisted of a portion of stomach and duodenum which measured 15 by 13 by 2 cm. An annular ulcer, 9 by 5 cm., was present in the prepyloric region. The base of the ulcer was firm, irregular, gray, and 2 cm. thick. The edges were slightly rolled and hyperplastic. The uninvolved wall of the stomach measured 1 cm. in thickness. Seventeen lymph nodes were found attached to the serosal surface of the specimen, the largest measuring 2 by 1 cm. They were firm and discrete and the cut surface was gray and homogeneous.

The entire wall of the stomach was infiltrated at the site of the ulcer by large mononuclear cells. However, they did not involve the serosa. Many small mononuclear cells also were found. It was estimated that 80 per cent of the infiltrating cells were plasma cells.

The lymph nodes retained their normal architecture. The sinusoids were distended with fluid, plasma cells, and a few leukocytes. Large numbers of plasma cells were also found in the stroma. The perinodal fat was moderately infiltrated by plasma cells.

The individual plasma cells were for the most part typical. They were oval to round cells with abundant cytoplasm which stained a homogeneous bluish red with hematoxylin and eosin, with an eccentrically placed nucleus with cartwheel formation of the chromatin material, a paranuclear vacuole in some cells, and a small centrally placed nucleolus. However, many plasma cells were not typical. Binucleated cells were frequent. Other cells, while conforming to the general structure of plasma cells, were not mature. They were larger than the adult form. The nucleus was likewise larger, with an increase in the nuclear-cytoplasmic ratio. The chromatin material was arranged in strands with irregular condensations around the periphery. Two nucleoli were common. These cells were considered immature plasma cells or plasmablasts.

Autopsy Report

Autopsy was performed 8 hours after death. Only the pertinent findings are included in this report. The margins of the upper one-third of the surgical incision were not completely approximated. This opening communicated with a small abscess cavity, 3 cm in diameter, in

the abdominal wall. No unusual findings were present at the site of resection.

An embolus was present at the bifurcation of the pulmonary arteries. It completely occluded the left branch, and partially occluded the right. There were several depressed, blue, firm areas in both lower lobes, which were considered to be due to atelectasis. The source of this embolus was not determined.

The axillary, thoracic, abdominal, and retroperitoneal lymph nodes were found to be enlarged, discrete, and soft. The largest measured 2 cm. in length and 1 cm. in diameter. Especially large nodes were found in the celiac and superior mesenteric groups, and around the head of the pancreas and in the gastrohepatic ligament. These nodes were in close approximation to the operative site. The adenopathy involved to some extent all periaortic and iliac groups and the nodes along the ascending colon. The thoracic nodes were enlarged, but not to a greater degree than is often found in routine autopsies.

Microscopic Features. The capillaries throughout the entire body were dilated and filled with erythrocytes. Albuminous fluid was present in the myocardium and in the pulmonary alveoli. Small focal areas of organizing pneumonia, large macrophages containing pigment, and a small healed infarct also were present in the lungs. There was a chronic inflammatory reaction with foreign body giant cells and organized thrombi in the small vessels at the operative site in the head of the pancreas. It was impossible to evaluate the plasma cells quantitatively in the various groups of nodes by any precise method. Since plasma cells are frequently found in nodes without any specific lesions, we used the axillary nodes as a norm, as they did not seem to have an abnormal number. The various sections of these nodes either contained no plasma cells or but a few in the stroma. This was interpreted as 1 plus. On this rough quantitative basis the nodes around the ascending colon were graded 2 plus, and the periaortic, celiac, and mesenteric nodes as 3 plus. The perigastric nodes removed surgically were 4 plus. The mesenteric nodes had plasma cells invading the capsule and extending into the perinodal fat. The mesenteric, celiac, and right axillary nodes were moderately scarred and edematous.

Anatomic Diagnoses. Plasmacytoma of stomach with lymphadenopathy of regional lymph nodes with infiltration of plasma cells; pulmonary embolus with complete occlusion of left and partial occlusion of right pulmonary artery; hyperemia of viscera; pulmonary edema and edema of myocardium; granulating surgical wound of abdominal wall; postoperative abdominal adhesions; small focal areas of organizing pneumonia; small pulmonary infarct.

DISCUSSION

The plasma cell apparently is a definite cytologic entity. Its origin and function are still the subject of much discussion. Michels,[3] in 1931, reviewed the theories of the morphogenesis, function, and developmental capacity of the plasma cell. In his review he gave the following concepts as to its origin: (1) from connective tissue cells, (2) emigrated lymphocytes, (3) monocytes or pre-existent tissue lymphocytes, (4) immature blood cells. Lowenhaupt,[4] recently, in studying cases of multiple myeloma, concluded that the plasma cells arise from tissue histiocytes of the spleen and lymph nodes. Plasma cells are frequently present in normal lymphatic tissue according to Maximow and Bloom.[5] In view of these theories as to the origin of the plasma cell, it seems reasonable to assume that a plasmacytoma could arise in the stomach.

Plasma cells are usually present to some degree in chronic pyogenic and granulomatous lesions. They may also form specific tumors, arising primarily in the bone marrow or viscera.[1,6] Some consider that this cell may characterize a leukemic state.[7-10] In our case the histologic picture did not resemble any granulomatous process with which we are familiar. No fungi or acid-fast bacilli were found. The Kahn test on the peripheral blood was negative. We concluded, therefore, that this was a primary plasmacytoma of the stomach. Dr. Shields Warren and Dr. C. A. Hellwig reviewed the slides and concurred in the diagnosis.[11,12]

The next problem was whether this tumor was benign or malignant. The patient gave a vague clinical history of gastric symptoms for 7 years, suggesting a benign lesion. The duration, however, of an extramedullary plasmacytoma is not a criterion for either its recurrence after removal or the presence of metastases. These tumors may have a duration of 10 or more years before metastasizing. There is evidence which leads us to conclude that the lesion was malignant. The immaturity and atypical appearance of the plasma cells, and their apparent invasive qualities are in keeping with a malignant process. However, Hellwig[1] pointed out that "the microscopic appearance does not play such a dominating rôle in predicting the clinical course of a given lesion. . . . . the localization and the gross appearance seem to be more reliable criteria than the histologic structure."

The lymph nodes around the stomach, removed at the time of resection, likewise bore out the assumption that the gastric lesion was malignant. The presence of large numbers of plasma cells in the peripheral sinuses and in the stroma is readily explained by spread from the primary lesion to the regional lymph nodes. The presence,

likewise, of plasma cells in the capsule and perinodal fat gave added weight to the conclusion that this is probably an invasive lesion. Since we can find no other case reports of lesions in this location, and since the patient died 14 days postoperatively, the possibility of recurrence could not be evaluated as a criterion of malignancy.

It is interesting to note that, as far as we are able to determine, this plasmacytoma was limited to the stomach and regional lymph nodes. We found no increase in number or abnormal types of plasma cells in distant nodes, bone marrow, or peripheral blood. Many writers [6-10] have called attention to the occurrence of coexisting plasmacytomas of the marrow or viscera, and a plasma cell leukemia. In spite of the 7-year history and the size of the primary lesion, no generalization had taken place. It is interesting to speculate, but impossible to prove, whether, had widespread involvement taken place, it would have been the result of metastasis or of multicentric origin of neoplastic plasma cells.

SUMMARY

A case of plasmacytoma of the stomach is presented. No similar case could be found in the literature. Involvement of regional lymph nodes and local infiltration indicated that this neoplasm was malignant at the time of operation.

REFERENCES

1. Hellwig, C. A. Extramedullary plasma cell tumors as observed in various locations. *Arch. Path.*, 1943, 36, 95–111.
2. Brown, C. R., and Liber, A. F. Multiple plasmoma of the ileum and colon. (Abstract.) *Arch. Path.*, 1939, 28, 112–113.
3. Michels, N. A. The plasma cell. A critical review of its morphogenesis, function and developmental capacity under normal and under abnormal conditions. *Arch. Path.*, 1931, 11, 775–793.
4. Lowenhaupt, E. Proliferative lesions in multiple myeloma with special reference to those of the spleen. The origin of the plasma cell. *Am. J. Path.*, 1945, 21, 171–185.
5. Maximow, A. A., and Bloom, W. A Textbook of Histology. W. B. Saunders Co., Philadelphia, 1942, ed. 4, pp. 60, 79, 80.
6. Geschickter, C. F., and Copeland, M. M. Multiple myeloma. *Arch. Surg.*, 1928, 16, 807–863.
7. Piney, A. Recent Advances in Hematology. P. Blakiston's Son & Co., Philadelphia, 1939, pp. 218–222.
8. Whitbey, L. E. H., and Britton, C. J. C. Disorders of the Blood. The Blakiston Co., Philadelphia, 1942, ed. 4, pp. 411–413.
9. Patek, A. J., Jr., and Castle, W. B. Plasma cell leukemia. A consideration of the literature with the report of a case. *Am. J. M. Sc.*, 1936, 191, 788–793.
10. Reiter, B. R., and Freeman, J. T. Plasma cell leukemia. *Am. J. M. Sc.*, 1937, 193, 38–42.
11. Warren, S. Personal communication.
12. Hellwig, C. A. Personal communication.

[Illustrations follow]

DESCRIPTION OF PLATE

PLATE 41

Fig. 1. Lymph node along the greater curvature of the stomach, showing plasma cells in dilated peripheral sinusoids and infiltrating into the perinodal fat. Hematoxylin and eosin stain. × 100.

Fig. 2. High-power magnification of the same node as depicted in Figure 1, showing typical plasma cells. Hematoxylin and eosin stain. × 450.

Fig. 3. Pylorus, showing part of the ulcerated plasmacytoma. The cross section is through the base of the ulcer which is about two times as thick as the uninvolved stomach wall.

Fig. 4. The wall of the ulcer, showing the cellular infiltration between the muscle bundles. These cells are almost entirely plasma cells. Hematoxylin and eosin stain. × 100.
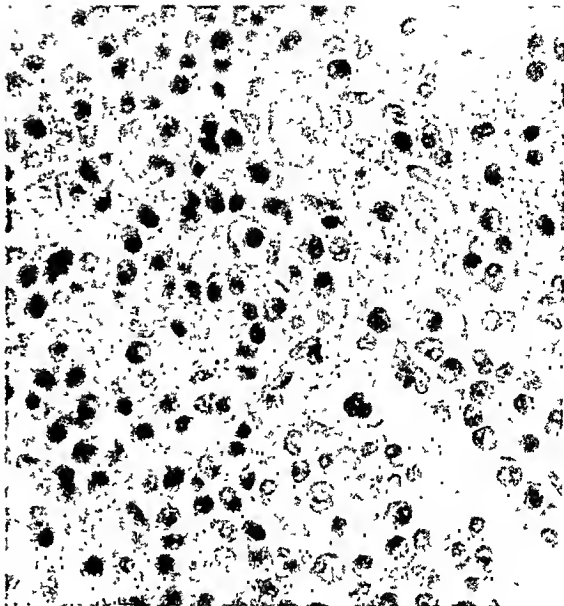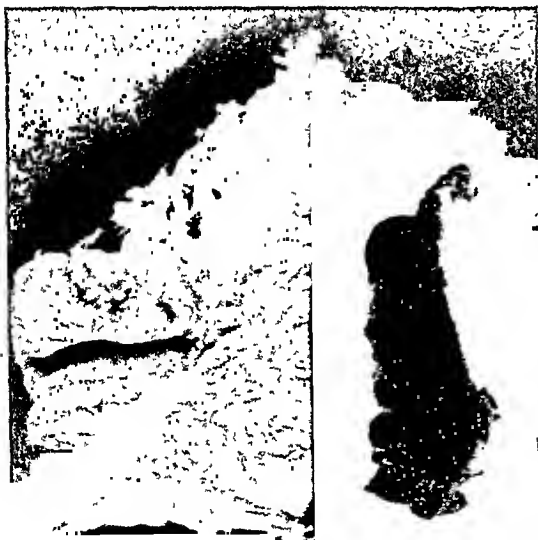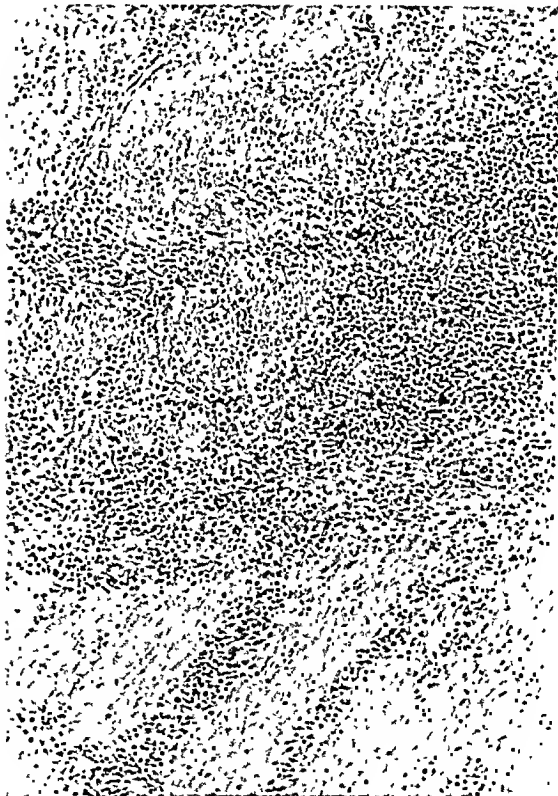
Schwander, Estes, and Cooper   Plasmacytoma of the Stomach

THE FATE OF CARCINOMA EMBOLI IN THE LUNG *

OTTO SAPHIR, M.D.

(From the Department of Pathology,† Michael Reese Hospital, Chicago 16, Ill.)

During the routine examination of histologic sections taken from lungs of patients dying from carcinoma of various organs, outspoken thickening of the intima of smaller arteries and veins was often noticed. This was found in lungs which disclosed metastatic tumors as well as in those in which no metastases were encountered. These changes were more severe and were much more frequently encountered in patients with carcinoma than in patients of similar or older ages without carcinoma. Soon it was noted that there was a relationship between such thickened blood vessels and the presence of remnants of tumor emboli. The purpose of this investigation was to study the fate of tumor emboli in the lungs and their relation to the seeming sclerosis of the smaller blood vessels of the lung.

This study is based on the histologic examination of the lungs of 12 patients dying from carcinoma of various sources. In 5 instances the primary carcinoma was in the colon, in 2 instances in the stomach, ovaries, and cervix uteri, and once in the skin. The lungs, grossly, showed no evidence of metastasis. The ages of the patients varied from 36 to 60 years. Only those lungs were studied which histologically disclosed tumor emboli but no frank metastasis. Numerous blocks were taken from such lungs and stained with iron hematoxylin and eosin, and with orcein for elastic fibers. Serial sections were taken from several blocks.

It has been known for many years that tumor emboli may occur in the lungs in the absence of metastasis. As early as 1903, Schmidt [1] found tumor emboli in the pulmonary arterioles in 15 instances, in only 5 of which tumor cells were encountered exclusively within the vessels. Evidence of degeneration of these tumor emboli was described. Since Schmidt's publication, similar observations have been recorded from time to time. Iwasaki,[2] who studied tumor emboli histologically, concluded that tumor emboli are often made innocuous by the process of organization. Takahashi [3] noted the gradual disappearance of tumor cells in the blood vessels of the lungs of mice which had been injected intravenously with tumor cells.

Willis [4] stated that "tumour embolism is not metastasis" and that "many neoplastic emboli perish or remain sterile in their new sites of arrest." He also gave a number of pertinent references. Warren and

Gates[5] studied the fate of intravenously injected tumor cells. They stated that, on the basis of their experimental studies, there is no doubt that tumor emboli may become organized, may disappear or remain latent over long periods without establishing true metastases. They left open the question whether this is the result of death of some of the cells, which initiates thrombus formation, or of mechanical factors, or of the resistance of the host.

In the following, a short description is given of changes within the walls of vessels which harbor tumor emboli.

Tumor emboli were found in the smaller and smallest branches of the pulmonary artery, in capillaries, and within the small pulmonary veins. In some instances the tumor cells were clumped, occupying only a relatively small portion of the lumen. Adjacent to these tumor cells were fibrin and many red blood corpuscles. In other sections tumor emboli filled the entire lumen on cross section. In some fields in which the vessel was only partially filled with tumor cells, hyaline thrombi were encountered close to the cancer cells and at a distance from them. These hyaline thrombi often were rather recent, and evidence of incipient organization was either lacking or very early. Here and there such hyaline emboli were completely or partially surrounded by newly formed lining endothelial cells. Often, tumor emboli and small hyaline thrombi were encountered side by side. In such instances it was occasionally noted also that the tumor emboli were separated from the remainder of the lumen of the vessel by one layer of lining endothelial cells. The cells constituting the tumor emboli varied, depending upon the morphologic characteristics of the primary tumor. Most commonly they consisted of individual epithelial cells, not arranged in any particular fashion even in those instances in which the primary tumors proved to be adenocarcinomas. Often the tumor cells showed oval, spindle-shaped, or round nuclei which were densely stained. Anachromasia and anaplasia were constant findings. It might be noteworthy to emphasize that the spindle-shaped tumor cells were found principally at the periphery of the tumor emboli, the nuclei in their centers being distinctly vesicular and more round.

In some sections it was noted that the tumor cells were obviously compressed by the hyaline thrombus which apparently grew by apposition of new thrombotic material. In obviously older instances, some of the tumor cells, particularly those located adjacent to the thrombi, seemingly were thinned out, had become atrophic, and finally were only indistinctly outlined. Gradually the hyaline thrombus seemed to extend into the region of the atrophic tumor cells which eventually were replaced by the thrombus. Often, masses of tumor cells were

divided into smaller accumulations and were separated from one another by thrombi. The impression was gained that the thrombus had burrowed between the clumps of tumor cells. It is interesting to note that also in these seemingly atrophic tumor cell masses, occasional atypical mitotic figures could still be recognized. Often, also, clumps of obviously compressed tumor cells were found completely encircled by a hyaline thrombus. Coincident with the growth of the thrombus and resulting organization, such clumps of tumor cells were sometimes surrounded by young connective tissue cells. Since sometimes the hyaline thrombus was at least partially surrounded by a layer of lining endothelial cells continuous with that of the vessel, cancer cells could often be noted in the small openings where the original thrombus had approximated the opposite wall. Thus the impression was gained that the thrombus was canalized, the individual channels being partially filled with cancer cells. In some of the fields adjacent to those that showed tumor emboli, hyaline thrombi in various stages of organization were often recognized completely occluding the lumina. Only rarely a few tumor cells were still recognized somewhere within the thrombus.

Remarkably often, small branches of the pulmonary arteries were encountered containing tumor cells intermixed with thrombi attached to the intima of only a relatively small portion of the blood vessel. Into such mural thrombi fibroblastic cells often were seen extending from the wall and gradually replacing the thrombus. Eventually such a thrombus was completely replaced by hyalinized connective tissue and covered by lining endothelial cells. Thus, the impression was often gained of an arteriosclerotic plaque rather than of an organized thrombus. Such plaques were found very often in the various sections taken from the lung. Occasionally within the same vessel an early organizing mural thrombus was noted upon which was superimposed a more recent tumor embolus.

DISCUSSION

Outstanding changes were encountered within the vessels which contained tumor emboli. The histologic study indicates that these emboli, when they fail to leave the capillaries and produce metastases, cause the formation of hyaline thrombi. These thrombi either surround the tumor emboli or are formed adjacent to them when tumor cells become adherent to the vessel wall. The hyaline thrombi seemingly cause atrophy of the adjacent tumor cells, and eventually autolysis, the tumor cells being gradually replaced by the growing hyaline thrombus. The thrombus becomes organized, but often tumor cells still are found in various portions of the lumen surrounded by the organized or organizing thrombus. Thus, occasionally, canalized thrombi

are seen with clumps of tumor cells within the channels. In instances in which only mural thrombi are found, the eventual organization causes localized intimal thickening which very closely resembles pulmonary arteriosclerosis. Such localized thickening of the smaller branches of the pulmonary arteries in patients who have a primary carcinoma seems to indicate that at some time previously pulmonary tumor emboli had been present.

From the foregoing, it seems clear that it is not the tumor emboli which become "organized," but that the hyaline thrombi which very likely had been caused by the tumor emboli gradually become organized. As these thrombi grow and become organized, the tumor cells gradually disappear and are replaced by the organizing thrombus.

Willis [4] raised the question whether blood or blood clot inhibits neoplastic growth. After citing some experimental work in regard to the fate of intravenously injected tumor cells within the pulmonary blood vessels, he recommended caution before attributing intravascular degeneration and death of such introduced tumor cells to any specific anti-cancerous or inhibitory quality residing in blood plasma or blood clot. Also, Warren and Gates [5] concluded that blood has no toxic effect on tumor cells.

As was pointed out before, tumor emboli and hyalinized thrombi were encountered in both arteries and veins. Thus it seems that neither presence nor lack of oxygen plays any rôle in destroying the tumor cells. From the histologic picture it is possible that it is the mechanical injury brought about by the thrombus with consequent compression of the tumor cells which is detrimental to the cancer cells. However, it is equally clear that this cannot be the only explanation since in other lungs which were the seat of metastases, organizing thrombi and tumor emboli were simultaneously encountered in the smaller blood vessels.

The tumor cells constituting the emboli are definitely viable. They stain well and even details of nuclear structures are often clearly visible. Mitotic figures are often observed. Also, that in serial sections tumor cells were found in the smallest arteries, capillaries, and veins may speak for the fact that these cells have eventually grown into the veins. Emboli consisting of nonviable tumor cells are bound to lodge in the smallest branches of the pulmonary artery and capillaries, and to remain there. Yet, as was brought out previously, the walls of the vessels were not invaded by tumor cells in these cases.

The localized and diffuse thickening of the intima of the smaller arteries is noteworthy. As a matter of fact, it is this type of vessel change which was noted first and which stimulated a detailed examination of the lungs in these instances. The intimal thickenings are

end-stages of completely organized thrombi. Although transitions are often encountered from hyaline thrombi with tumor cells to organizing thrombi with tumor cells, in such end-stages cancer cells were never seen. It is clear that diffuse thickening of the intima, the result of organized mural thrombi, closely resembles and, as a matter of fact, often cannot be distinguished from pulmonary arteriosclerosis. This is found so frequently that it seems justified to suspect cancer emboli in the lungs of patients with cancer when histologic sections disclose unexplained arteriosclerosis of the pulmonary arterial branches.

SUMMARY

Twelve lungs in which carcinoma emboli were encountered on routine histologic examination were studied. These emboli were found in the smaller and smallest branches of the pulmonary artery, in capillaries, and in small pulmonary veins. A small sheath of fibrin and hyaline thrombi almost invariably were encountered adjacent to the tumor cells. The thrombi seemingly caused atrophy of adjacent tumor cells which were gradually replaced by the growing thrombi. Eventually these thrombi became organized, but clumps of tumor cells were still recognizable either within the new channels or embedded within the growing connective tissue. Thus, not the tumor emboli but the hyaline thrombus became organized and caused the disappearance of the tumor cells. When only mural thrombi were encountered, the eventual organization caused localized intimal thickening which very closely resembled pulmonary arteriosclerosis.

REFERENCES

1. Schmidt, M. B. Die Verbreitungswege der Karzinome und die Beziehung generalisierter Sarkome zu den leukämischen Neubildungen. G. Fischer, Jena, 1903.
2. Iwasaki, T. Histological and experimental observations on the destruction of tumor cells in the blood vessels. *J. Path. & Bact.*, 1915–16, 20, 85–105.
3. Takahashi, M. An experimental study of metastasis. *J. Path. & Bact.*, 1915, 20, 1–13.
4. Willis, R. A. The Spread of Tumours in the Human Body. J. & A. Churchill, London, 1934.
5. Warren, S., and Gates, O. The fate of intravenously injected tumor cells. *Am. J. Cancer*, 1936, 27, 485–492.

[*Illustrations follow*]

DESCRIPTION OF PLATES

PLATE 42

FIG. 1. Tumor cells and fibrin intermingled in a small pulmonary artery. Iron-hematoxylin and eosin preparation. × 180.

FIG. 2. Carcinoma cells and fibrin in pulmonary vein. Iron-hematoxylin and eosin preparation. × 230.

FIG. 3. Of note are the tumor cells, hyaline thrombus, and organizing thrombus. Hematoxylin and eosin preparation. × 180.

FIG. 4. Hyaline thrombus with very early organization and tumor cells in a pulmonary vein. Iron-hematoxylin and eosin preparation. × 90.

FIG. 5. Organizing thrombus with superimposed carcinoma embolus. Iron-hematoxylin and eosin preparation. × 180.

FIG. 6. Small pulmonary artery with hyaline thrombus in the center and tumor emboli at both sides of the hyaline thrombus. Iron-hematoxylin and eosin preparation. × 90.

1
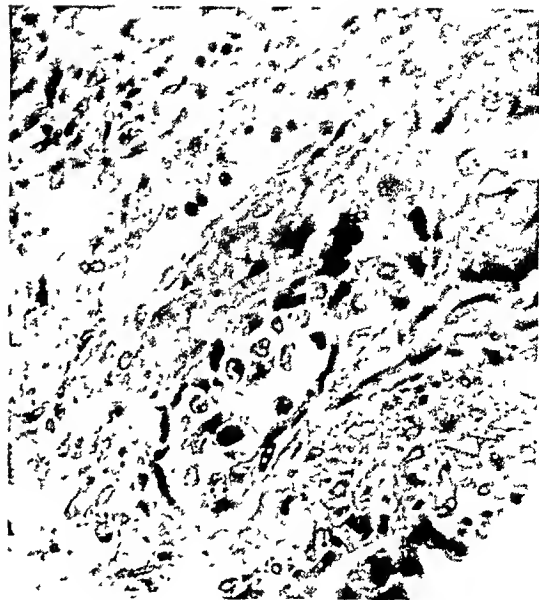
2

3
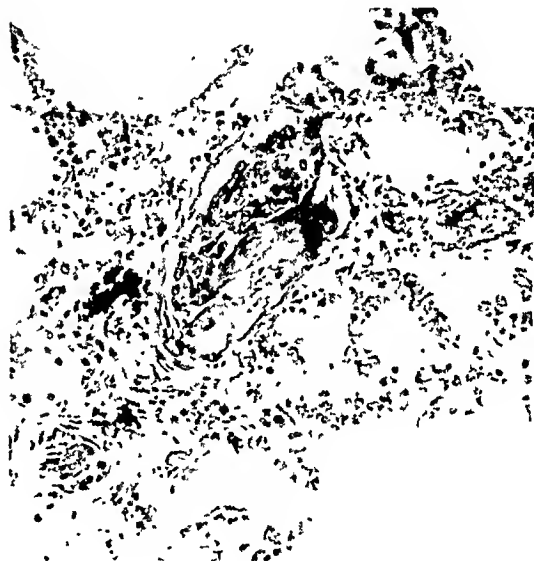
4

5
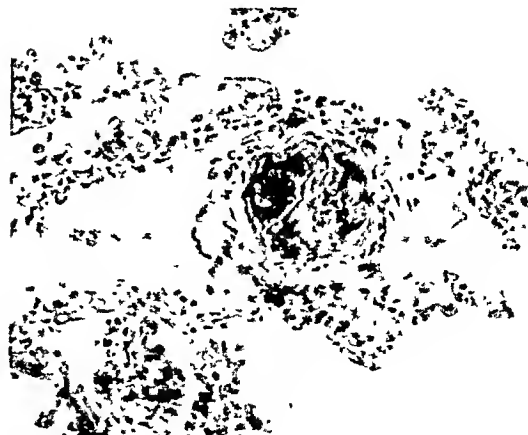
6

Saphir

Carcinoma Emboli in the Lung

PLATE 43

FIG. 7. Organizing thrombus with superimposed carcinoma embolus. Of note are the endothelial cells lining the thrombus. Hematoxylin and eosin preparation. × 90.

FIG. 8. Hyaline thrombus with remnants of tumor cells. Hematoxylin and eosin preparation. × 90.

FIG. 9. Organizing thrombus with superimposed hyaline thrombus and carcinoma embolus between. Iron-hematoxylin and eosin preparation. × 180.

FIG. 10. Transformation of organizing thrombus into a knob-like intimal thickening. Iron-hematoxylin and eosin preparation. × 180.

FIG. 11. Thick-walled small pulmonary artery resembling arteriosclerosis. Hematoxylin and eosin preparation. × 180.

7

8
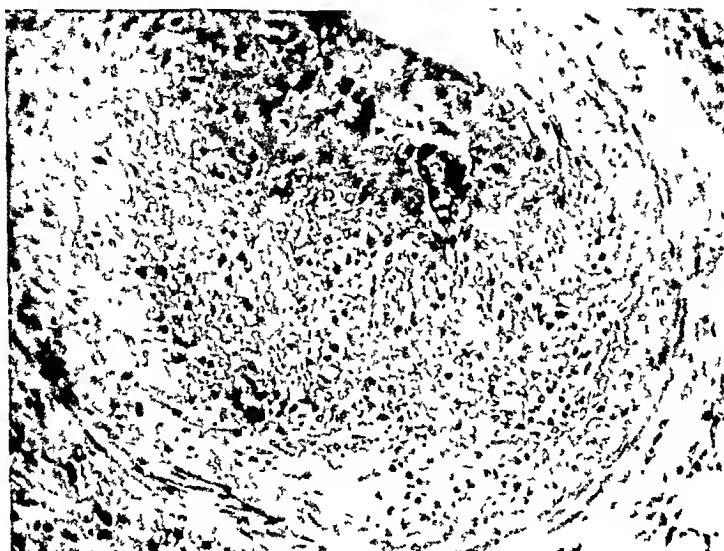
9

10

11

Saphir   Carcinoma Emboli in the Lung

THE PERMEABILITY OF THE RENAL GLOMERULI OF SEVERAL MAMMALIAN SPECIES TO LABELLED PROTEINS *

Hans Smetana, M.D.

(*From the Department of Pathology, College of Physicians and Surgeons, Columbia University, New York 32, N.Y.*)

While studying the origin of colloid droplets in urodeles, it was found that intravenous injection of proteins, labelled with diazotized dyes, was followed by the appearance of tiny granules, colored by these dyes, in the lining cells of the convoluted tubules of both the "closed" and "open" nephrons.[1] Because of this unanticipated finding, a systematic study was made to investigate the permeability of the renal glomerular filter of several mammalian species to labelled proteins.

Materials and Methods

The various proteins to be used were coupled with the disodium salt of 2-naphtol-3 : 6 disulfonic acid (R salt) according to procedures described by Kabat and Heidelberger.[2] Nitrogen determinations were made with the micro-Kjeldahl method in duplicate. Three times re-crystallized egg albumin was prepared according to Heidelberger.[3] Purified solutions of serum albumin and serum globulin were prepared in the usual manner.

The preparations used in the experiments were as follows:

Neopeptone (Difco Laboratories, Detroit, Michigan)-R salt
Egg albumin (thrice re-crystallized)-R salt
Serum albumin (cat, dog, mouse)-R salt
Serum globulin (cat, dog, mouse)-R salt
Diazotized R salt alone, 0.12 per cent.

The different dilutions of these protein-dye compounds are given in the reports of the respective experiments.

Procedures

White mice, white rats, albino guinea-pigs, albino rabbits, and mongrel dogs were injected intravenously with varying amounts of the above-mentioned protein-R salt preparations and were allowed to live for periods of time ranging from 30 minutes to 28 days after the injection. During this time, they were fed on the regular stock diets used for these various species.

After autopsy, the organs were fixed immediately in Zenker's fluid and in a 4 per cent solution of formaldehyde. The paraffin sections were examined either unstained but cleared, or stained with hematoxy-

lin only, or stained with hematoxylin and light green in order to provide a color contrast. The protein-R salt compounds present in the tissues remained unaltered for years and could easily be identified, even in minute amounts, by their intense bright red color.

The Distribution of Protein-R Salt Compounds in the Organs after Intravenous Injection

Mouse Series 1

Groups of 5 mice, each weighing 30 gm., were injected with 0.5 cc. of protein-R salt preparations and the animals were sacrificed 30 minutes, 1 hour, 2 hours, 3½ hours, and 24 hours after a single injection.

(a) Peptone-R salt, 0.2 mg. of N per cc. (21.7 mg. of protein per kg. of body weight). The findings in the organs were as follows: The Kupffer cells showed a faint reddish tint in animals killed 1 hour after the injection; definite red granules were present in these cells 3½ to 24 hours after the injection, and similar granules were found in a few reticulo-endothelial cells of the spleen. The urine of all animals had a definite reddish tint. Sections of the kidneys showed no trace of red matter.

A repetition of the experiment using a concentration of 0.3 mg. of N per cc. (27 mg. of protein per kg. of body weight) gave similar results. Peptone preparations proved to be toxic to mice so that injections had to be given slowly and in small portions.

(b) Egg albumin-R salt, 1.1 mg. of N per cc. (114.5 mg. of protein per kg. of body weight). The Kupffer cells and some of the reticulo-endothelial cells in the spleen and in various other viscera showed granules and small clumps of bright red matter 30 minutes after the injection. The lining cells of the proximal portion of the renal proximal convoluted tubules, including some of the lining cells of the spaces of Bowman, contained tiny bright red granules situated within the cytoplasm (see Figs. 1 and 2). These granules became more definite and abundant the longer the animals lived after the injection, and they appeared to be present in all of the lining cells of the proximal convoluted tubules. They were never observed in any other portion of the renal tubular system.

(c) Serum albumin (cat)-R salt, 1 mg. of N per cc. (104 mg. of protein per kg. of body weight). The results of these experiments were similar to those observed in (b), except that granules in the lining cells of the convoluted tubules were first observed in animals sacrificed 3½ hours after the injection. They were more numerous and definite after 24 hours.

(d) Serum globulin (cat)-R salt, 2 mg. of N per cc. (208 mg.

of protein per kg. of body weight). A few red granules were present in the Kupffer cells only 30 minutes after the injection; 1 hour after the injection similar granules were seen also in the reticulo-endothelial cells of various viscera and tiny red dots made their appearance in the lining cells of the proximal convoluted tubules of the kidneys. These were more numerous and definite in mice which had been allowed to live a longer period of time after the injection.

Summary of Results of Mouse Series 1. Intravenous injection of protein-R salt compounds was followed by the appearance of labelled materials in the reticulo-endothelial cells of the viscera and within the lining cells of the proximal convoluted tubules of the kidneys where they appeared from ½ to 3½ hours after the injection.

Mouse Series 2

Groups of 8 mice each were injected intravenously on 2 successive days with 0.5 cc. of various protein-R salt compounds and pairs of animals of each group were sacrificed 2, 4, 13, and 28 days after the first injection.

(a) Egg albumin-R salt, 1.1 mg. of N per cc. (229 mg. of protein per kg. of body weight). In sections from animals killed 2 days after the injection, all of the lining cells of the proximal convoluted tubules contained abundant cytoplasmic red granules. The Kupffer cells, reticulo-endothelial cells, and interstitial cells of the viscera were laden with clumps and granules of red matter. Similar changes were present in the sections from mice 4 days after the administration of protein-R salt compounds. Thirteen days after the injection, only occasional clumps of granules were left in the lining cells of the convoluted tubules while masses of red matter were present in the Kupffer cells and reticulo-endothelial cells. In animals which lived 28 days after the injection, scarce small granules remained in the lining cells of the tubules while interstitial cells, Kupffer cells, and reticulo-endothelial cells contained ample red material. There was sloughing and degeneration of lining cells of the convoluted tubules so that detached cells, containing red granules, could often be seen within the lumina.

(b) Serum albumin (dog)-R salt, 0.72 mg. of N per cc. (150 mg. of protein per kg. of body weight). The findings were similar to those in (a) except that practically no granules were found in the lining cells of the renal tubules 13 and 28 days after the injection.

(c) Serum globulin (dog)-R salt, 1.17 mg. of N per cc. (244 mg. of protein per kg. of body weight). The findings were similar to those described above. Thirteen days after the injection, only a few of the

lining cells of the convoluted tubules showed groups of granules, which were rather coarse. Most of the interstitial cells exhibited phagocytized red matter and the reticulo-endothelial cells contained masses of red material. Twenty-eight days after the injection no more granules could be found in the lining cells of the renal tubules.

Summary of Results of Mouse Series 2. The number of granules within the lining cells of the renal convoluted tubules decreased steadily the longer the animals were allowed to live after the injection of protein-R salt compounds. There was desquamation of these lining cells so that only scarce red dots remained 28 days after the injection of egg albumin-R salt and serum albumin-R salt; none were seen in mice 28 days after the injection of serum globulin-R salt. The Kupffer cells, reticulo-endothelial cells, and interstitial cells of the viscera retained the material very well.

Mouse Series 3

Two groups of 6 mice each were injected intravenously one to five times on successive days with 0.3 cc. of homologous mouse serum protein preparations coupled with R salt. Two animals of each group were sacrificed 24 hours after the first injection and one animal of each group was killed 24 hours after each following injection. In addition, 2 mice were injected intravenously with diazotized R salt alone and were sacrificed 24 hours afterwards.

(a) Serum Albumin (Mouse)-R Salt, 0.5 mg. of N per cc.

(1) Twenty-four hours after the administration of 31.3 mg. of protein-R salt per kg. of body weight, some of the Kupffer cells showed a faint reddish tint and capillaries of the lungs contained reddish casts. No other changes were recognized.

(2) After intravenous administration of 63 mg. of serum albumin-R salt per kg. of body weight, abundant bright red granules were visible in the lining cells of the proximal convoluted tubules in addition to those present in the reticulo-endothelial cells and interstitial cells of the viscera.

(3) However, 24 hours after the injection of 68 mg. of serum albumin-R salt per kg. of body weight, the results were similar to those described in (a) (1). No granules were seen in the renal tubules.

(4) The results seen after the injection of 94, 125, and 156 mg. of serum albumin-R salt per kg. of body weight were similar to those described under (a) (2). The number of granules present in the lining cells of the tubules was roughly proportional to the amount of material administered (Figs. 1 and 2).

(b) Serum Globulin (Mouse)-R Salt, 0.56 mg. of N per cc.

(1) After the injection of 35.4 mg. of protein-R salt per kg. of body weight, only the Kupffer cells showed a faint reddish tint. No granules were seen in the kidneys.

(2) Injection of 50 mg. of serum globulin-R salt per kg. of body weight was followed by the appearance of a few faintly reddish granules in the lining cells of the renal proximal convoluted tubules; the Kupffer cells contained faintly stained reddish masses.

(3) After injection of 71 mg. of protein-R salt per kg. of body weight, the granules in the kidneys were definite but scarce, while those in the reticulo-endothelial cells were numerous.

(4) After intravenous administration of 106, 142.2, and 174 mg. of homologous serum globulin per kg. of body weight, abundant granules were seen in the tubular lining cells as well as in the reticulo-endothelial cells and interstitial cells of various viscera.

(c) Diazotized R Salt Alone, 0.12 per cent (12 mg. of R Salt
per kg. of Body Weight)

Very few tiny red granules were present in the lining cells of the proximal convoluted tubules while numerous granules were apparent in the reticulo-endothelial cells and in some of the interstitial cells of the viscera.

Summary of Results of Mouse Series 3. Intravenous injections of protein preparations from homologous species coupled with R salt were followed by the appearance of phagocytized materials in the reticulo-endothelial cells of the viscera as well as in the lining cells of the renal proximal convoluted tubules. The number of granules depended on the amount of material administered. Injections of diazotized R salt alone produced similar results.

Rat Series

Three groups of 2 rats each, weighing, on the average, 150 gm., were injected intravenously with 3 cc. of protein-R salt compounds and the animals were sacrificed 24 hours later.

(a) Egg albumin-R salt, 1.69 mg. of N per cc. (211.3 mg. of protein per kg. of body weight). Tiny bright red granules were present in the lining cells of the proximal convoluted renal tubules, and the reticulo-endothelial cells of the viscera contained phagocytized red matter.

(b) Serum albumin (dog)-R salt, 1.83 mg. of N per cc. (229 mg. of protein per kg. of body weight). The results of these experiments were similar to those of (a).

(c) Serum-globulin (dog)-R salt, 0.95 mg. of N per cc. (119 mg.

of protein per kg. of body weight). The results of these experiments were similar to those described above, but occasional pale red granules were seen in a few lining cells of the proximal convoluted tubules.

Summary of Results of Rat Series. After intravenous injection of protein-R salt compounds into rats, phagocytized red matter was seen in the reticulo-endothelial cells of the viscera and tiny red granules were present in the lining cells of the proximal convoluted tubules.

Guinea-Pig Series

Three groups of 2 guinea-pigs each, weighing, on the average, 250 gm., were injected intravenously with from 2.5 to 4 cc. of protein-R salt compounds and the animals were sacrificed 4 hours after the injection; 2 animals were injected with diazotized R salt alone.

(a) Egg albumin-R salt, 1.69 mg. of N per cc. (102.5 mg. of protein per kg. of body weight). Some of the Kupffer cells in the liver showed a faint red coloration. There was no trace of the labelled protein in any of the other viscera.

(b) Serum albumin (dog)-R salt, 1.83 mg. of N per cc. (183 mg. of protein per kg. of body weight). The results were similar to those of (a).

(c) Serum globulin (dog)-R salt, 0.95 mg. of N per cc. (71.3 mg. of protein per kg. of body weight). The results of these experiments were similar to those of (a).

(d) Diazotized R salt alone, 0.12 per cent (14.4 mg. of R salt per kg. of body weight). No colored particles were seen in any of the viscera. R salt alone, given intravenously, proved to be quite toxic to guinea-pigs; therefore, injections had to be given slowly and in small portions.

Summary of Results of Guinea-Pig Series. With the exception of Kupffer cells, which showed a slight reddish coloration, there was no evidence of the presence of labelled proteins in any of the viscera after intravenous injection of protein-R salt compounds.

Rabbit Series

(a) One albino rabbit, weighing about 2000 gm., was injected 16 times with 1 cc. each of an egg albumin-R salt preparation containing 0.8 mg. of N per cc. A total amount of 40 mg. of protein per kg. of body weight was given and the animal was sacrificed 1 month after the first injection and 5 days after the last administration. In microscopic sections of the viscera, red granules were present in the reticulo-endothelial cells of the viscera but not elsewhere.

(b) One albino rabbit, weighing approximately 2000 gm., was

injected 16 times with 1 cc. each of a solution of diazotized R salt alone. A total amount of 9.6 mg. of R salt per kg. of body weight was given. The result was similar to that described above.

Summary of Results of Rabbit Series. There was no evidence of the presence of labelled materials in the lining cells of the renal tubules after intravenous injection of egg albumin-R salt or of diazotized R salt alone. The reticulo-endothelial cells of the viscera, however, contained phagocytized red particles.

Dog Series 1

(a) A healthy dog, weighing 10.2 kg., was injected intravenously with 65 cc. of a solution of egg albumin-R salt, containing 1.69 mg. of N per cc. A total amount of 67.3 mg. of protein per kg. of body weight was given and the animal was sacrificed 24 hours afterwards. Microscopic sections showed tiny red granules in the lining cells of the renal proximal convoluted tubules and in the reticulo-endothelial cells of the viscera.

(b) A healthy dog, weighing 12.8 kg., was injected intravenously with 120 cc. of diazotized R salt alone. A total of 11.3 mg. of R salt per kg. of body weight was given and the animal was sacrificed 24 hours later. Sections of the organs showed granular red matter in the reticulo-endothelial cells of the viscera but not elsewhere.

Summary of Dog Series 1. After intravenous injection of egg albumin-R salt, colored particles appeared in the reticulo-endothelial cells of the viscera and in the lining cells of the proximal convoluted tubules. Although red granules were present in the reticulo-endothelial cells after intravenous injection of diazotized R salt alone, the renal tubules showed no evidence of the presence of colored matter.

Dog Series 2

In order to study the renal excretion of R salt after damage to the kidneys, 3 dogs received 4.0, 6.0, and 8.0 mg., respectively, of a solution of uranium nitrate subcutaneously[4] before intravenous injections of diazotized R salt. After the applications of uranium nitrate the urine contained ample albumin.

(a) A healthy dog, weighing 11 kg., was injected subcutaneously with a solution of uranium nitrate; a total amount of 4 mg. of uranium nitrate per kg. of body weight was given. Fifty cc. of a 0.12 per cent solution of diazotized R salt were given intravenously 43 hours after the administration of uranium nitrate and, similarly, 100 cc. of R salt were injected 53 hours later. A total amount of 16.4 mg. of R salt per kg. of body weight was injected, and the animal was sacrificed 4 hours

after the last, and 54 hours after the first, injection of the R salt. At autopsy, the urine showed a faint reddish tint. Microscopic sections of the organs disclosed granular red matter in the reticulo-endothelial cells of the viscera. There was extensive necrosis of the lining cells of the convoluted tubules and within these cells were seen faintly red-stained masses of irregular sizes, but no well formed granules.

(b) A healthy dog, weighing 9.5 kg., was given 6 mg. of uranium nitrate per kg. of body weight subcutaneously. Three days afterwards 100 cc. of diazotized R salt were injected intravenously and 150 cc. were given again the following day. A total amount of 31.6 mg. of R salt per kg. of body weight was administered. The animal died 15 minutes after the last injection. At autopsy, the liver was large and dark red; the gallbladder was distended by abundant bile; the urinary bladder contained urine showing a faint red color. Microscopic sections showed changes similar to those described under (a). The lumina of the convoluted tubules were distended by protein casts exhibiting a faint reddish hue.

(c) A healthy dog, weighing 10.5 kg., was injected with 8 mg. of uranium nitrate subcutaneously, and 100 cc. of diazotized R salt were given 3 days later and again the following day. A total amount of 22.8 mg. of R salt was administered and the animal was sacrificed 5 hours after the last injection. Microscopic studies of the viscera showed results similar to those described above.

Summary of Results of Dog Series 2. After damage to the kidneys by uranium nitrate followed by intravenous injection of diazotized R salt, ill defined, faintly red-stained masses were found in some of the necrotic lining cells of the renal convoluted tubules; however, no well formed granules were seen. Colored particles were present in the reticulo-endothelial cells of the viscera.

DISCUSSION

The presence of labelled protein particles within the lining cells of the renal convoluted tubules after intravenous injection of various protein-dye compounds indicated that such substances were able to pass the glomerular filter. This conception hinges on the validity of the assumption that the diazotized dyes are inseparably coupled with the protein molecule serving as a label by which the presence of the respective protein can be recognized. This was proved chemically by Kabat and Heidelberger[2] and by Smetana and Johnson[1] in electrophoretic experiments.

To what extent the proteins are denatured by coupling them to the R salt is not known. In anaphylactic experiments with guinea-pigs,

using native proteins for sensitization, and proteins coupled with R salt for the final injection or vice versa, no differences were observed; likewise qualitative as well as quantitative precipitin reactions with either coupled antisera against native antigen or vice versa gave results identical to those with controls.[5]

The amount of protein passing through the normal glomerular filter is probably too small to be detected by ordinary laboratory methods, while even minute amounts of protein-R salt preparations can be visualized microscopically. The filtering membrane apparently does not differentiate between foreign or homologous species proteins. No studies were made with homologous serum proteins coupled with R salt; however, it is assumed that diazotized R salt injected into the blood stream combines with the serum proteins, thereby forming labelled homologous serum protein· compounds. In mice, these compounds did pass through the glomerular filter, as the presence of tiny red granules within some of the lining cells of renal convoluted tubules indicates.

The passage of protein substances of relatively large molecular sizes, such as serum albumin and serum globulin, through the normal glomerular filter is rather surprising and changes to some extent the physiologic conception of the function of the filtering membrane of the renal glomerulus. However, it has been shown that even under normal condition some protein is regularly escaping into the glomerular filtrate from which it is absorbed by the lining cells of the proximal convoluted tubules.[6]

The appearance of labelled protein particles within the lining cells of the proximal convoluted tubules strongly suggests that they are absorbed by these cells from the glomerular urine. Although secretion of these substances by the lining cells has to be considered, this seems unlikely because the granules first make their appearance in the lining cells of the funnels of the convoluted tubules or even in the lining cells of the spaces of Bowman before they can be seen in the supraglomerular loops, but are never found in cells of any other portion of the renal tubular system. If they were excreted by the lining cells of the tubules, one would expect colored material in the urine to appear for some time after the injection, which is not the case. It is realized, however, that only direct observation of the nephron during an acute experiment can settle this problem definitely.

The fate of the granules within the lining cells of the tubules is linked with the length of life of the cells in which they are situated: their number diminishes, due to desquamation of lining cells, until none or very few are left. In mice this takes about 1 month after the last

administration of protein-R salt compounds. The phagocytized particles of labelled protein compounds in the Kupffer cells, reticulo-endothelial cells, and in the phagocytic interstitial cells of various viscera remain indefinitely.

The failure to demonstrate labelled protein granules within the lining cells of renal tubules in experiments with guinea-pigs and rabbits perhaps indicates species differences. However, when the amount of injected protein-dye compounds per kg. of body weight is computed, it appears likely that too little material was given to rabbits, so that the results obtained in this series are inconclusive (Table I). Similarly,

TABLE I

Tabulation of Results to Show Quantitative Level at Which Granules Appeared in the Renal Tubular Epithelium

Animals	Mg. of substrate per kg. of body weight				
	R salt	Peptone	Egg-albumin	Serum-albumin	Serum-globulin
Mouse series	12.0	21.7	114.5	31.3	35.4
Mouse series		27.0	229.0	63.0	50.0
Mouse series				68.0	70.8
Mouse series				93.8*	106.0*
Rat series			211.3	229.0	119.0
Guinea-pig series	14.4		102.5	183.0	71.3
Rabbit series	9.6		40.0		
Dog series	11.3		67.3		

Underscored figures indicate granules in renal tubules; figures not underscored indicate no granules in renal tubules.

* Amounts larger than these always gave positive results.

the results obtained in mice with peptone-R salt preparations are inconclusive; due to the toxicity of this preparation, larger amounts were not tolerated.

In general, it can be stated that the greater the amount of protein-R salt given, the more extensive were the deposits found in the lining cells of the renal convoluted tubules as well as in the reticulo-endothelial cells of the various viscera. A minimal amount of about 60 mg. of protein per kg. of body weight has to be administered before granules appear in the lining cells of the kidney tubules (Table I).

After intravenous injection of diazotized R salt alone into dogs following damage of the kidneys due to uranium nitrate, small amounts of this dye passed through the glomerular filter and faintly stained masses of red substance were seen in some of the necrotic lining cells of the convoluted tubules. This is in contrast to the brilliantly stained, well defined granules which appear in the tubular cells of normal animals injected with protein-R salt compounds. It is suggested that

the formation of these granules is an expression of a normally functioning cell and this has perhaps a bearing on the interpretation of colloid droplets as being protein particles stored in functioning lining cells of tubules after re-absorption from tubular lumina of protein substances which have passed through the glomerular filter.

CONCLUSIONS

1. Preparations of egg albumin, and of serum albumin and serum globulin of heterologous and homologous species, labelled by R salt, pass the glomerular filter of normal mice, rats, and dogs after intravenous injection; particles of these substances are re-absorbed by the lining cells of the proximal convoluted tubules after passage through the glomerular filter and are stored in these cells in the form of tiny granules.

2. The number of granules present in the lining cells of the tubules is roughly proportional to the amounts of protein-dye compounds administered.

3. The particles of protein-dye compound remain in the lining cells of the tubules until these cells are desquamated.

REFERENCES

1. Smetana, H., and Johnson, F. R. The origin of colloid and lipoid droplets in the epithelial cells of the renal tubules. *Am. J. Path.*, 1942, 18, 1029–1049.
2. Kabat, E. A., and Heidelberger, M. A quantitative theory of the precipitin reaction. V. The reaction between crystalline horse serum albumin and antibody formed in the rabbit. *J. Exper. Med.*, 1937, 66, 229–250.
3. Heidelberger, M. Advanced Laboratory Manual of Organic Chemistry. Chemical Catalog Co., New York, 1923, p. 83.
4. MacNider, W. de B. Urine formation during the acute and chronic nephritis induced by uranium nitrate. A consideration of the functional value of the proximal convoluted tubule. *Harvey Lectures*, 1928–29, 24, 82–105.
5. Smetana, H. Unpublished data.
6. Oliver, J. New directions in renal morphology: a method, its results and its future. *Harvey Lectures*, 1944–45, 40, 102–155.

[Illustrations follow]

PLATE 44

FIG. 1. Mouse series 3 (five intravenous injections on successive days of homologous mouse serum albumin-R salt, 0.5 mg. of N per cc., totalling 156 mg. of protein per kg. of body weight). Section of kidney, showing a glomerulus, the space of Bowman, and the funnel of the proximal convoluted tubule. The tiny black dots within the cytoplasm of the lining cells of the convoluted tubule and in some of the cells lining the space of Bowman represent brilliantly stained red granules of the serum albumin-R salt. × 1050.

FIG. 2. Section of kidney from the same animal as shown in Figure 1. The small black dots in the cytoplasm of the lining cells of the proximal convoluted tubules represent the intensely stained red granules of the serum albumin-R salt. × 1050.
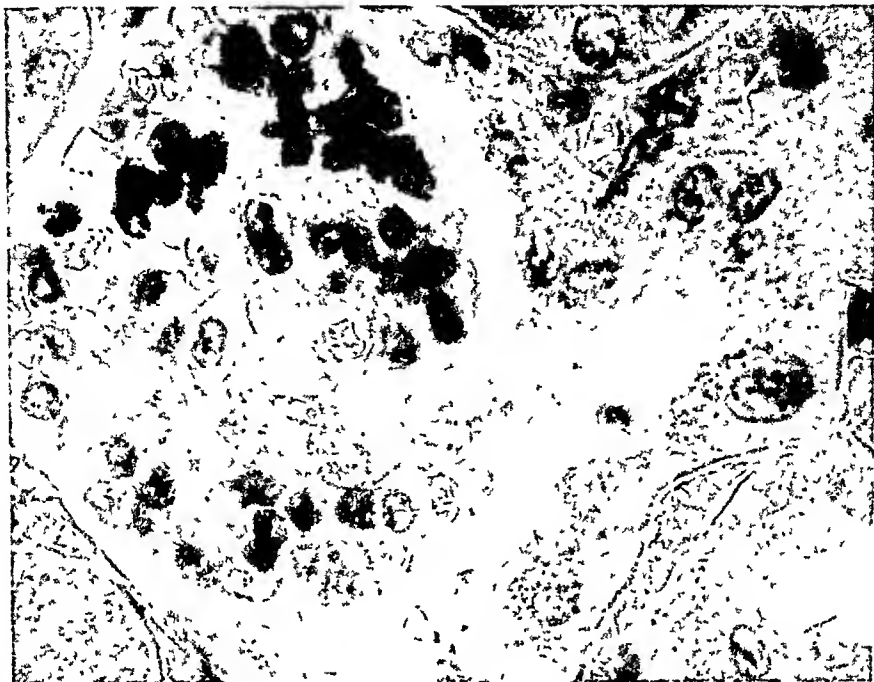
1
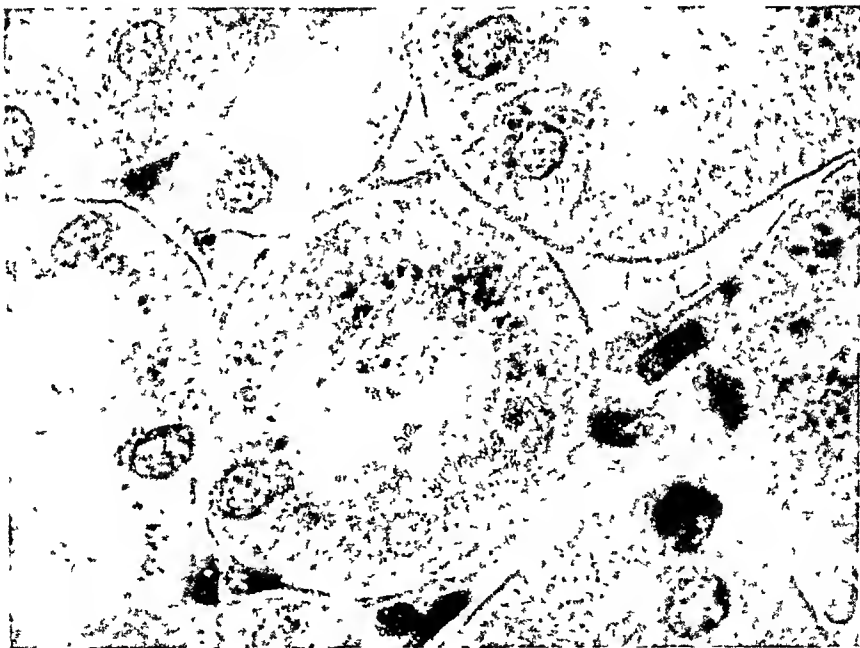
2

INTRALOBULAR REGENERATION OF LIVER CELLS IN MAN *

C. T. Ashworth, M.D., and H. C. Reid, M.D.

(*From the Department of Pathology of Southwestern Medical College and Parkland Hospital, Dallas, Texas*)

Regeneration of liver tissue is a well recognized phenomenon, having been described in 1833 by Cruveilhier.[1] Much experimental work has been done in an attempt to elucidate the mechanisms concerned. Most of these observations have been made in connection with regeneration which follows partial hepatectomy, and that which results from extensive chemical injury of the liver cells. Ponfick,[2] in 1889, clearly demonstrated the phenomenon of regeneration after hepatectomy, and many others have contributed similar information in more recent years (Milne,[3] Fishback,[4] Brues, Drury, and Brues,[5] Sulkin[6]). The matter of mitotic or amitotic division of cells in the regenerating liver[7] and the actual means of enlargement of the regenerating lobule (Mann[8]) still appear to be somewhat controversial. Few factors which regulate or influence liver cell regeneration have been determined. Mann and Magath[9] demonstrated the necessity of an intact portal circulation, and Brues, Drury, and Brues found that diet exerted an important influence on regeneration.

In the study of human material one has frequent opportunity to note extensive liver cell regeneration, particularly in cirrhosis and in instances of acute and subacute necrosis of more or less extensive form produced by a variety of agents. Our attention has been drawn recently to the fact that definite evidence of regeneration of liver cells without nodule formation exists with great frequency in human livers in which neither cirrhosis nor extensive liver cell necrosis has been present. Regeneration of this type has been largely neglected in previous studies, and it is with this type that the present study is concerned.

METHODS OF STUDY

A series of 100 autopsy cases was studied without selection except that the records of a few infants were discarded so that they would not constitute too large a proportion of the group. Also, cases of cirrhosis were excluded. The clinical records and autopsy reports were consulted for significant findings. The principal study consisted of a histological investigation of the livers in which observations on the presence, degree, morphological characteristics, and distribution of the regenerative phenomena were made, and other lesions in the liver described. This report deals with an analysis of these histological findings as related to certain clinical and gross anatomical features.

INCIDENCE

In order to determine the frequency of the aforementioned histological changes in routine autopsy cases it was necessary to establish a baseline of normal histological appearance of the liver. Even in cases of accidental death the liver sometimes showed slight to moderate regeneration. More or less arbitrarily, we have decided to classify as essentially within normal limits those livers in which there was either no evidence whatever of regeneration or only very rare areas of modified, regenerating cells. Thirty-seven per cent, according to these criteria, were normal livers as far as regeneration was concerned, while 30 per cent showed 1 plus; 21 per cent, 2 plus; 6 per cent, 3 plus; and 6 per cent, 4 plus regeneration.

TABLE I

Distribution as to Age of Livers Showing Evidence of Regeneration

Age	Total no.	No. of cases without regeneration	No. of cases with regeneration			
			+*	++	+++	++++
(years)						
Below 1	8	7	1	0	0	0
1–10	2	2	0	0	0	0
11–20	7	6	0	0	1	0
21–30	5	2	2	1	0	0
31–50	26	14	5	5	1	1
51–70	35	5	17	9	2	2
71–90	17	1	5	6	2	3

* An arbitrary quantitative differentiation of the regeneration, based on the experience in this series of cases.

Age

It was found that evidence of regeneration appears much more commonly in adults than in infants and children. However, definite regeneration, never to a marked degree, was occasionally noted in the young. The distribution according to age is given in Table I.

MORPHOLOGICAL CHARACTERISTICS

Cells revealing evidence of regeneration were modified in appearance with respect to both nucleus and cytoplasm. The most characteristic change consisted of enlargement of the nuclei. These often were found to be one and one-half times the normal diameter and sometimes as much as three times the normal. The nuclei showed somewhat greater variation in shape than is normal, often being oval, but usually they were rounded, and sometimes quite irregular. These enlarged nuclei were characterized, further, by an increased amount of chromatin. Nucleoli were more prominent than usual, increased in number to five or six, and almost always larger than the normal liver cell

nucleoli. Many of these hyperchromatic and enlarged nuclei were double, that is, the liver cells were binucleate.

The cytoplasm was increased in amount, although some exceptions to this were noted. The cytoplasm was usually more opaque, and rather paler than normally. Fat droplets and bilirubin pigment were noted often in the cytoplasm of regenerating cells. Occasionally, areas of regeneration were characterized by a syncytium-like arrangement, and the cells did not form discrete cords. Usually, however, large liver cords were formed by the cells, or the regenerating cells occupied positions in otherwise normal-appearing cords.

Concerning the distribution of the areas of regeneration, considerable variation existed. Usually they were focal, either in the center or periphery of the lobule. In every case the lobular architecture of the liver was retained. The designation "intralobular regeneration" would seem advisable in order to distinguish this process from the nodular regeneration of cirrhotic and post-necrotic livers. In those instances in which local retrogressive lesions were present in the liver, the regenerating cells were located in the area of the lesion or immediately around it. In several instances, especially when no other histological abnormalities were noted in the liver, the process of regeneration was diffuse throughout the lobule. Binucleate liver cells were more numerous in areas of regeneration, but only in one case were mitotic figures observed, and in this instance they were very numerous.

The hypertrophied nuclei of regenerated liver cells were frequently found to contain rounded, pale, eosinophilic inclusions similar to those noted by Andrew, Brown, and Johnson.[10] They, also, often contained fat droplets and bile pigment granules. The amount and appearance of the fat and bile coincided with that noted in the normal liver cells in the remainder of the section.

Association with Other Pathological Changes

The majority of cases of focal regeneration were found in patients who had chronic passive hyperemia of the liver. There were some cases, however, of long-standing hyperemia of the liver with atrophy of cells in the lobular center in which no regeneration was present. In those in which regeneration occurred, it was present largely in the zone of liver cells just peripheral to the atrophic or necrotic zone. Of 25 cases of chronic passive hyperemia with central atrophy or necrosis, 19 showed evidence of regeneration, the majority of which were 1 plus (Fig. 1). Of 8 cases of acute hyperemia, only one showed regeneration; this case was graded 1 plus.

There were 18 cases in which some degree of fatty metamorphosis

of the liver cells was encountered. In 5 there was no evidence of regeneration while in the remaining 13 cases 1 to 2 plus regeneration was found (Fig. 2). If any correlation existed between the degrees of fatty infiltration and of regeneration, it was an inverse correlation. The regeneration was found to involve cells containing fat droplets about as often as those which were free from fat. Binucleate cells were also noted among the fat-containing cells. Regeneration was accentuated in the cells around the foci of fatty infiltration.

In one group of cases there was marked emaciation. Usually, in this group, the livers were smaller than normal. There were 17 such cases, and of these there was no regeneration of the liver cells in 4. In 13 there was well marked regeneration. The liver cells which were not involved in the regenerative process were somewhat smaller than is normal and the cytoplasm darker. In these cases no other pathological changes were noted in the liver.

In the absence of other factors, infectious diseases did not appear to be associated with regeneration of liver cells.

In the 2 cases of eclampsia, regeneration was marked. There were 2 cases of central necrosis due to toxic agents, and in these regeneration was very marked.

There were 26 cases in which no factors were known, or hepatic lesions recognized, which might have a bearing on the incidence of regeneration. Of these, 14 cases showed no evidence of regeneration. These constituted a large portion of the normal livers of this series. In the remaining 12 some degree of regeneration was present. Interestingly enough, most of the cases of 4 plus regeneration fell in this category (Fig. 3). In some of these cases the regenerative activity was essentially diffuse in distribution, producing considerable enlargement of the lobules. The architecture of the lobules, however, was never disturbed.

Size of Nuclei of Regenerating Cells

Beams and King [11] and Sulkin [6] have recently described the nuclear changes in the regenerative cells of the liver of the partially hepatectomized rat. They noted marked increase in the size of nuclei and suggested that such hypertrophied nuclei were due to polyploidy. This conclusion was reached in view of the fact that peaks of incidence of liver cells of various sizes occurred in such a way as to indicate the presence of nuclei of two, four, eight, etc. times the volume of normal nuclei.

With this possibility in mind for human liver undergoing regenera-

tion, we have plotted the size of liver cell nuclei in normal and in regenerating livers. This was accomplished by the projection of cells at a standard magnification and drawing the nuclei directly on the image. From 100 to 200 nuclei, without selection, were drawn in each case studied and the drawings then measured.

The nuclei of normal human liver fall in a narrow range and are characterized by a single peak as to size (Text-Fig. 1). In those instances in which regeneration has occurred, the curve of nuclear size extends over a wide range, shifting markedly to larger sizes, with several small peaks of the nuclei of large sizes occurring in each case (Text-Fig. 2). It appears that these peaks fall at such intervals that, by calculation, the volume of the nuclei is roughly twice or four times
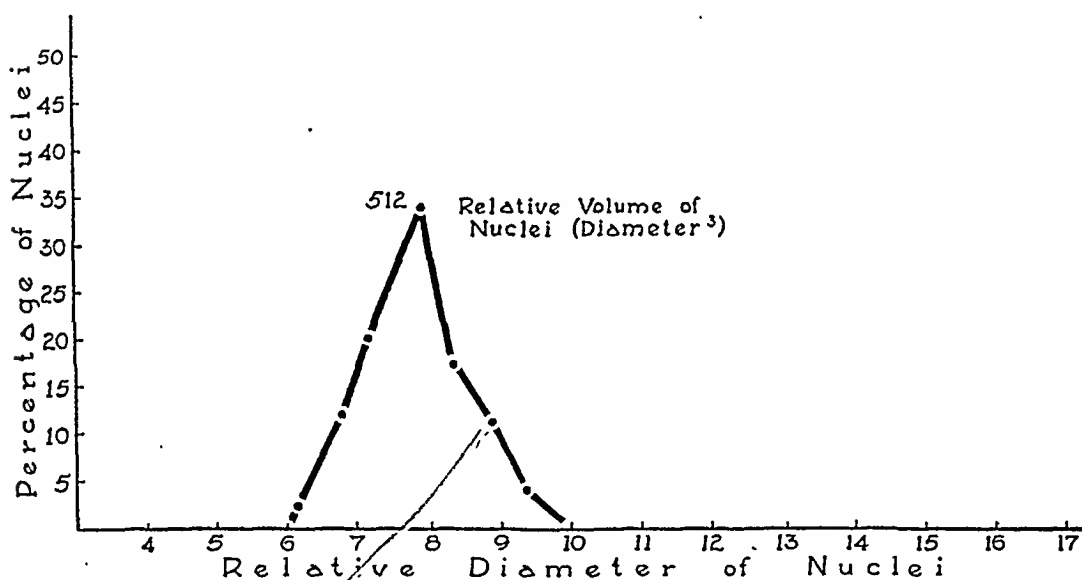
Text-Figure 1. Curve showing distribution of nuclear size in a normal liver.

the normal size. According to this criterion then, one might assume that polyploidy occurs in regeneration of human liver cells. According to Beams and King,[11] this process involves the development of binucleate cells, fusion of chromosomes of the two nuclei at a single mitotic spindle, and subsequent division with the formation of two cells, the nucleus of each having twice the normal chromosome component, that is, being tetraploid. Repeated mitosis with failure of cytoplasmic division, according to Beams and King, occurs in the rat, with the formation of octaploid, 16-ploid, etc. nuclei. Our findings would suggest a similar occurrence in man except for the almost complete absence of mitotic figures in the human liver undergoing regeneration. This matter will be considered further under the next topic.

Binucleate Cells

Cells containing double nuclei are well known to occur in the normal liver. Sulkin [6] found them to be increased in the rat in livers which were undergoing regeneration following partial hepatectomy. In the human liver without evidence of regeneration we have found binucleate cells to constitute about 4 per cent of the cells of the periphery of the lobule and 7 per cent at the center. In regenerating livers the percentage of binucleate cells averaged 11 and 14 per cent, respectively, at the periphery and center of the lobules. Binucleate cells were more numerous in the area where regeneration was active. In addition to binucleate cells, multinucleated liver cells containing as many as five or six nuclei were noted occasionally in the areas of regeneration. Most of the nuclei of binucleate cells were of about normal size, *i.e.*, diploid, but occasionally they were tetraploid. Mitotic figures were never en-
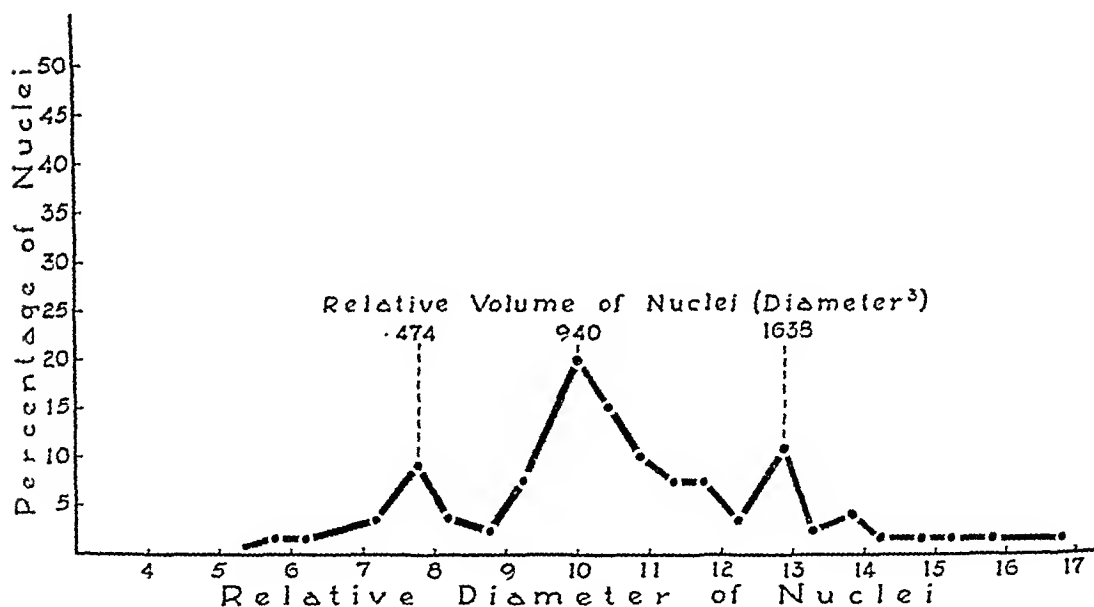
Text-Figure 2. Curve showing distribution of nuclear size in marked intralobular regeneration of the liver. 76-year-old male with no other lesions in the liver.

countered in binucleate cells, but every stage of what appeared to be amitotic division was found. This consisted of slight elongation, constriction at the middle of the elongated nucleus, and almost complete separation of the two segments of the dividing nucleus. The cytoplasm of cells with nuclear division proceeding in this manner was often eosinophilic and the same feature was noted in some cells in which the two nuclei were separate. In one case in which there was very active regeneration, mitotic figures were present in large numbers (Fig. 4). They occurred in areas where there was other evidence of regeneration, and in no instance were double nuclei encountered in the cells under-

going mitosis. If the mechanism of polyploidy occurs in the binucleate cells, however, single mitotic figures would be expected even if the cells were previously binucleate.

Regeneration without Local Lesions in the Liver

A remarkable phenomenon noted in several cases was that of marked diffuse regeneration without evidence of liver cell injury. Grossly, there were no changes in these livers except in a few in which faintly demarcated, small nodules could be made out on the cut surface; some were described as having a lighter brown, somewhat yellowish color. Microscopically, such livers showed on careful study that the faint nodular outlines were simply enlarged, rounded lobules with essentially normal architecture. The cells were very large and the nuclei prominently exhibited the changes listed as characteristic of regeneration. Occasionally, larger poorly outlined nodular areas seemed to compress adjacent liver tissue.

Analysis of the cases in which this appearance was noted did not reveal the cause of the regeneration. It seems probable that some form of mild active liver injury was occurring in these cases, perhaps in the nature of a dietary injury, which exaggerated the normal process of necrobiosis of liver cells and hence increased the rate of their new formation, producing the picture of regeneration.

DISCUSSION

Andrew, Brown, and Johnson [10] described hypertrophied and hyperchromatic nuclei in the livers of senile rats and men. As far as we can determine, the appearance of cells here described is similar to that described by them. It is possible that regeneration in senile animals occurs more commonly than in younger animals. MacNider [12] noted that regeneration in the young animal occurs readily with the formation of cells morphologically similar to the normal cells while regeneration in the older animal is characterized by the formation of larger cells which are more resistant to injury. Our observations are in accord with those of MacNider in this respect. Hypertrophied and hyperchromatic nuclei, although occurring occasionally in the young, are rare. It might be assumed that regeneration of liver cells in the older age group is characterized by polyploidy occurring in many of the dividing cells, and to a greater degree than is encountered in younger persons. Another factor to be taken into consideration in explaining the difference in incidence of regeneration in the young and old age groups is that those factors which seem to be associated with the occurrence of regeneration; namely, chronic passive hyperemia, fatty

infiltration, and emaciation, are seen far more commonly in older persons.

The mechanism of regeneration of liver cells has been the source of considerable controversy. Milne[3] found no evidence of participation of bile ducts. He explained the presence of small, solid bile duct "buds" as due to the fact that the connecting ducts between canaliculi and interlobular ducts are rendered more prominent through collapse. He considered newly formed cells to arise from other liver cells. Our findings are in complete accord. In our cases of focal regeneration there seems to be no question of the exclusive origin of regenerated cells from other liver cells. It appears that regeneration may proceed in any portion of the lobule. In instances of central liver cell injury, the regeneration develops in this region and in other cases the location of the injury determines the position of the regeneration. In view of the greater number of binucleate cells around the central vein as compared to the periphery, one may assume regeneration to occur more readily around the center, as Sulkin[6] indicated in his study of regeneration in the rat.

Regeneration in the liver might conceivably proceed through mitosis or amitosis. With the exception of one case, we noted no evidence of mitosis whatsoever. However, in this one instance it is certain that regeneration was largely through this mechanism. Beams and King,[11] and Sulkin[6] considered mitotic division to be the sole mechanism of regeneration in the rat. We found abundant morphological evidence suggesting amitotic division in practically every case of regeneration in man and are led to believe that this is by far the most important means of regeneration in the human liver, but that regeneration by mitotic division also may occur.

In these instances of regeneration it was noted that the lobular architecture of the liver was well preserved. This would seem to indicate that there is little likelihood of this process passing into cirrhosis. Destruction of total lobules or more extensive intralobular destruction with subsequent regeneration, on the other hand, characteristically may lead to portal cirrhosis.

Many local lesions of the liver which lead to atrophy, degeneration, or necrosis are followed by an appropriate regenerative response. In some cases, however, when regeneration was absent, there was evidence of destruction and injury of the liver cells. Such cases were marked by the rapid occurrence of death after the onset of the disease. This was found to occur particularly with acute hyperemia and degeneration of the liver cells. The greater the duration of injury, the more marked was the regeneration.

In several cases of extreme fatty infiltration, no evidence of regeneration was found. Some of these patients, however, died as a result of hepatic failure, and since the process was of many days' duration, it is believed that such altered liver cells may be incapable of regeneration.

SUMMARY

As opposed to nodular regeneration in the human liver following extensive necrosis and in cirrhosis, focal or diffuse intralobular hyperplasia is a very common finding in autopsy material. In a series of 100 cases this type of regeneration was found in 63 per cent. The prominent characteristics of this process consist of hypertrophy and hyperchromatism of nuclei, increased numbers of binucleate liver cells, and increased amount of cytoplasm.

The lesions in the liver commonly found to be associated with regeneration of this type were chronic passive hyperemia, fatty infiltration, and senile and malnutritional atrophy. Regeneration was found also in marked degree in connection with eclampsia and in a group of elderly patients without other apparent causes. In this latter group some of the most advanced degrees of intralobular regeneration were encountered.

Although mitotic figures were not observed in these regenerating areas of liver tissue, there was evidence of polyploidy, a phenomenon of karyokinesis occurring in binucleate cells. Thus, the increase of nuclear volume in the regenerating cells as compared to normal cell nuclei approached two to one or four to one.

It appears that in spite of the occurrence of advanced degrees of intralobular liver regeneration in many of these cases, no evidence of impaired function of the liver presents itself. Nor is there any suggestion that the lesion may proceed to the nodular type of regeneration characteristic of cirrhosis. On the other hand, the phenomenon is considered to be an expression of continuous and active cell replacement in the liver, which is speeded up in instances in which there is excessive intralobular cell loss, and which is characterized morphologically by the occurrence of polyploid nuclei and increased numbers of binucleate cells.

REFERENCES

1. Cruveilhier, J. Cited by Milne.[3]
2. Ponfick, E. Experimentelle Beiträge zur Pathologie der Leber. *Virchows Arch. f. path. Anat.*, 1889, 118, 209–249.
3. Milne, L. S. The histology of liver tissue regeneration. *J. Path. & Bact.*, 1908–09, 13, 127–160.
4. Fishback, F. C. A morphologic study of regeneration of the liver after partial removal. *Arch. Path.*, 1929, 7, 955–977.

5. Brues, A. M., Drury, D. R., and Brues, M. C. A quantitative study of cell growth in regenerating liver. *Arch. Path.*, 1936, 22, 658–673.

6. Sulkin, N. M. A study of the nucleus in the normal and hyperplastic liver of the rat. *Am. J. Anat.*, 1943, 73, 107–125.

7. Brues, A. M., and Marble, B. B. An analysis of mitosis in liver restoration. *J. Exper. Med.*, 1937, 65, 15–27.

8. Mann, F. C. Restoration and pathological reactions of liver. *J. Mt. Sinai Hosp.*, 1944, 11, 65–74.

9. Mann, F. C., and Magath, T. B. The production of chronic liver insufficiency. (Abstract.) *Am. J. Physiol.*, 1922, 59, 485.

10. Andrew, W., Brown, H. M., and Johnson, J. B. Senile changes in the liver of mouse and man, with special reference to the similarity of the nuclear alterations. *Am. J. Anat.*, 1943, 72, 199–221.

11. Beams, H. W., and King, R. L. The origin of binucleate and large mononucleate cells in the liver of the rat. *Anat. Rec.*, 1942, 83, 281–297.

12. MacNider, W. deB. A consideration of the acquired resistance of fixed tissue cells to injury. *Ann. Int. Med.*, 1938, 12, 147–165.

DESCRIPTION OF PLATES

PLATE 45

Fig. 1. Liver showing chronic passive hyperemia with moderate intralobular regeneration. Numerous binucleate cells with dark, eosinophilic cytoplasm. Hypertrophied, hyperchromatic nuclei. × 600.

Fig. 2. Liver with fatty infiltration, showing slight regeneration. × 600.

1

2

Ashworth and Reid
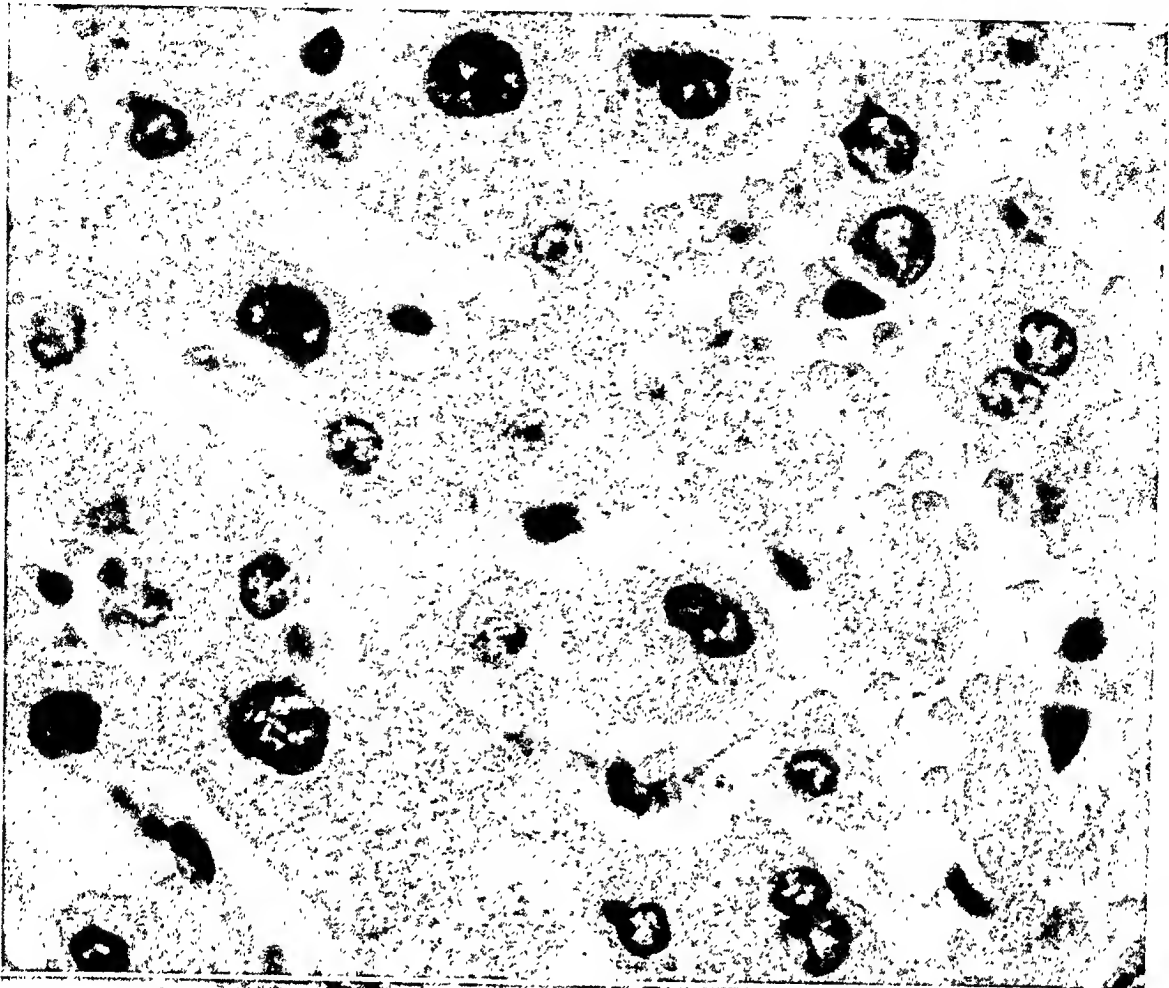
Regeneration of Liver Cells
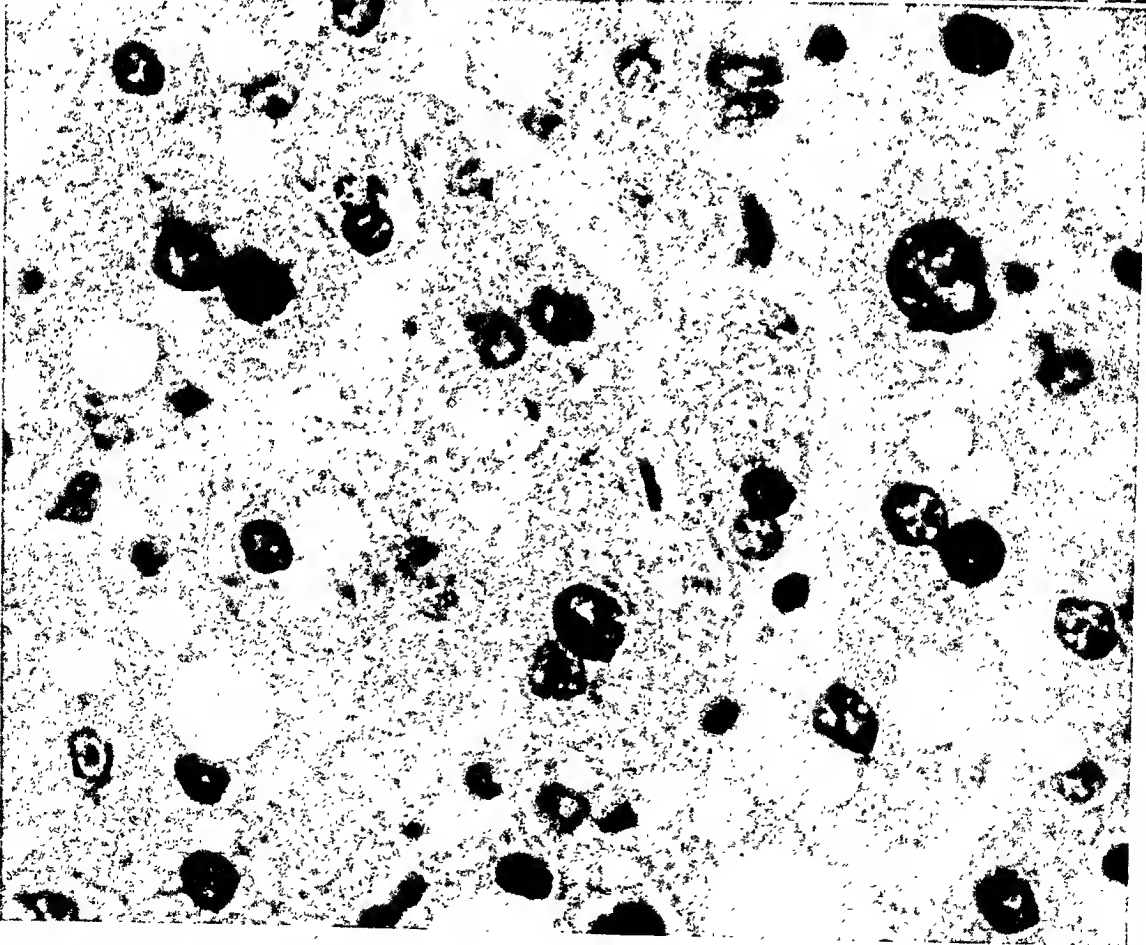
FIG. 3. Marked intralobular regeneration in a man, 76 years old. There were no other lesions in the liver. $\times$ 600.

FIG. 4. Moderate regeneration of the liver with numerous mitotic figures. One mitotic figure has an atypical, bifid spindle. $\times$ 600.

Ashworth and Reid

Regeneration of Liver Cells
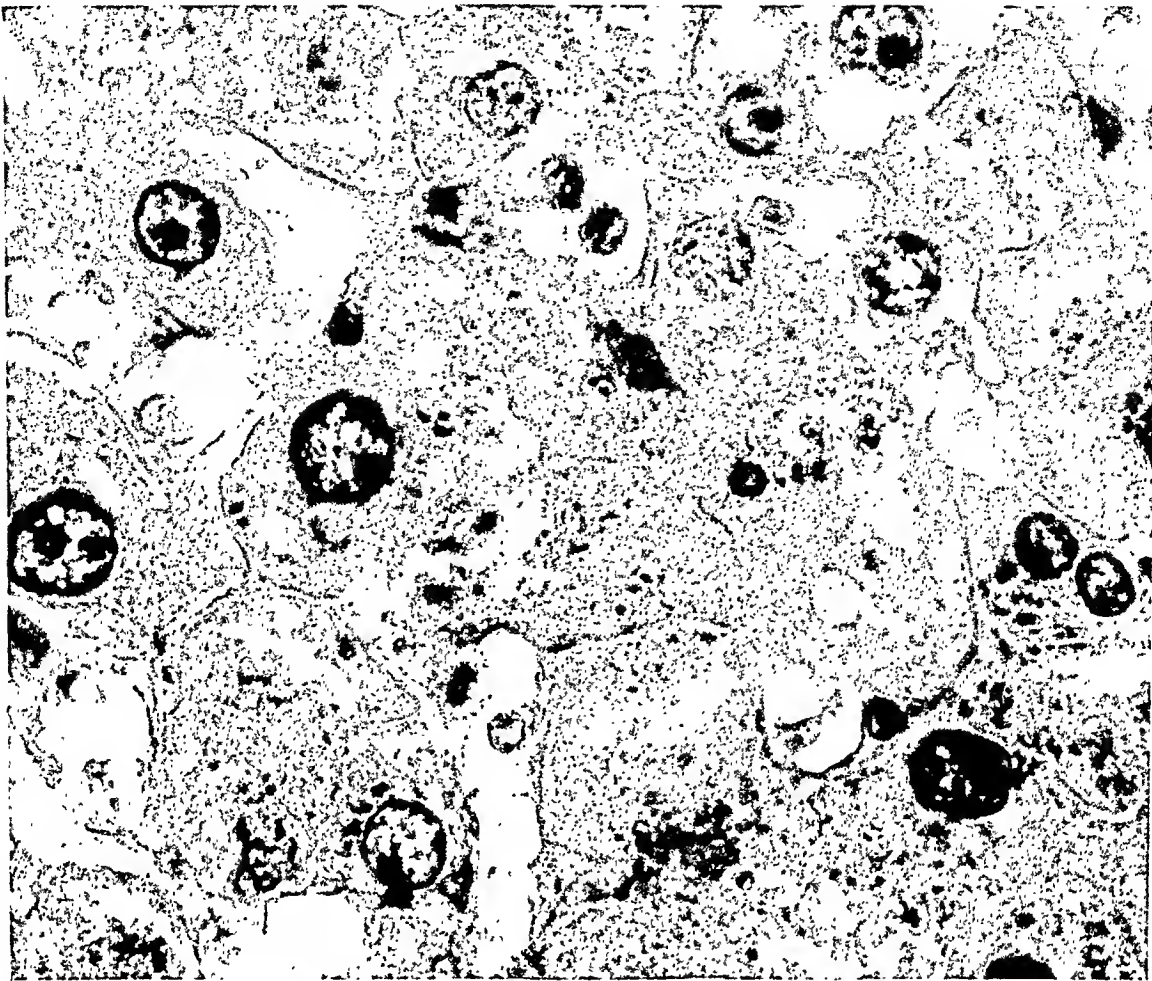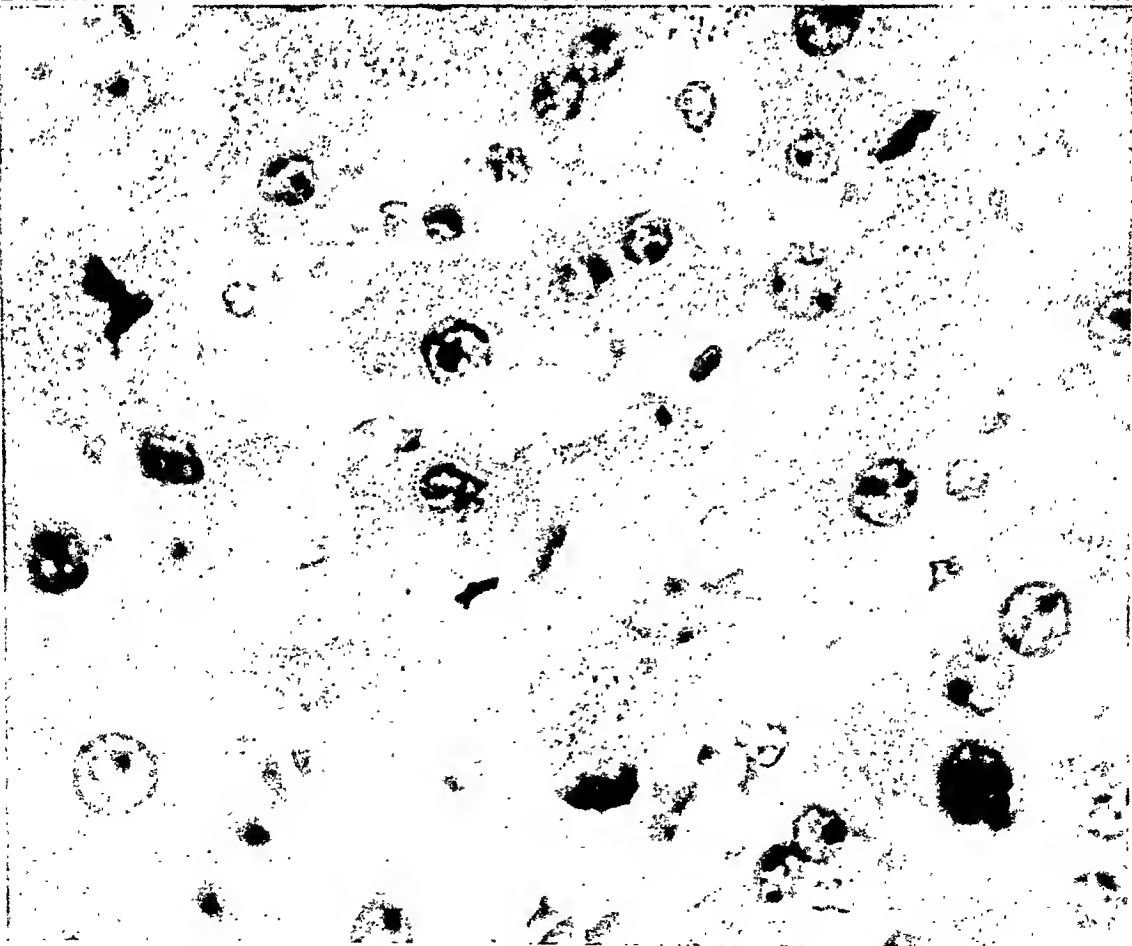
GIANT CELL TUMOR OF BONE

A Critical Survey *

ERNEST E. AEGERTER, M.D.

(*From the Department of Pathology, Temple University Hospital and School of Medicine, Philadelphia 40, Pa.*)

The giant cell tumor of bone with its curious cytologic structure has stimulated the imagination of a great number of writers. Indeed, a few hours with the literature on the lesion impresses one with the volume and variety of the conflicting ideas which have been expressed as to its nature. Theoretical observations concerning the lesion are hardly justifiable unless the author intends to simplify its definition. Such is the purpose of this discussion. Because this tumor is comprised of cells of two morphologically different types, it is set apart from most other neoplasms. I believe it is because these cells are found in other lesions, the nature and behavior of which are so different, that the enigma of the giant cell tumor has defied a universally satisfactory explanation.

The lesion has been described as a reparative reaction to injury. It has been explained as a granulomatous response to inflammation. Thus some writers have denied its inclusion among the tumors. On the other hand, among those who believe it to be of neoplastic character there is scarcely more accord. Geschickter and Copeland,[1] for example, stated that the tumor is a neoplastic proliferation of embryonic osteoclasts, while numerous other writers, among whom Jaffe, Lichtenstein, and Portis [2] are prominent, believe the tumor is essentially a fibrous growth characterized by the presence of the less important giant cells. The giant cells have been described as wandering phagocytes, as endothelial derivatives, and as megakaryocytes, but to the best of my knowledge they have always been considered an intrinsic part of a specific pathologic entity.

Not until comparatively recent years have three important bone diseases been described and set apart. These lesions are hyperparathyroidism and its distinction from the solitary bone cyst, fibrous dysplasia, and eosinophilic granuloma. Because all of these conditions may present a cytologic structure of giant cells in a fibrous matrix, they were almost certainly confused with giant cell tumors in the earlier literature.

In the light of accumulated knowledge there seem to be certain indisputable features of the usual concept of the giant cell tumor of bone. First of all the growth is composed of spindle cells which are of mesenchymal origin, among which are found multinucleated giant

cells and, much less frequently, large cells with a vacuolated cytoplasm described as foam cells containing lipid. It occurs predominantly in the third and fourth decades of life in the epiphyses and adjoining metaphyses of long bones. It is a neoplasm since it produces new tissue, often recurs, and may metastasize.[3] The relationship of this particular tumor to the other lesions characterized by a fibrous ground substance and giant cells will be discussed. This relationship becomes more understandable if one can accept certain theories concerning the genesis of the cells which constitute these lesions.

True adult fibrous connective tissue is probably only one of several specialized products which arise from a common ancestral tissue represented in postnatal life by mesenchyme. Other derivatives of the same stem material are bone, cartilage, fat, and the reticulum. Mesenchyme persists throughout life and serves, among other functions, as a mother substance for the regeneration of new tissue or the replacement of destroyed tissues which lack the potentiality of regeneration. From it fibroblasts arise and produce supportive and scar tissue. Chondroblasts and osteoblasts also stem from this source to produce their respective adult analogues. From reticulum probably come the myeloid elements of bone marrow itself.[4] Cells of phagocytic function commonly termed monocytes, phagocytes, or histiocytes are now generally assumed to arise from reticulum.[5] Indeed, the fibroblast itself is endowed with phagocytic potentialities. The histologist admits the difficulty and sometimes impossibility of differentiating the macrophage and the young fibroblast on a morphologic basis.

The multinucleated giant cells found in a variety of conditions and locations throughout the body offer interesting material for speculation.[6] The large cell which is produced in the reaction engendered by a foreign body is thought to be the product of a fusion of several macrophages which are commonly found in the region. The Langhans' cell of tuberculous lesions may have the same origin, but it is found among epithelioid cells which are probably nothing more than macrophages or fibroblasts arranged in a characteristic pattern. It is interesting to note that neither of these cells displays much phagocytic activity as demonstrated by ingested particles. It is as though they had satisfied their phagocytic purpose in swallowing each other or fusing to form one cell body with only the separate nuclei to testify to their original identity.

The osteoclast, another multinucleated giant cell, has been the vortex of a maelstrom of contention among pathologists writing on the pathology of bone. There has never been any considerable unanimity

concerning its origin, but its function now quite clearly seems to be different or at least qualified from that originally ascribed to it by von Kölliker.[7] A ponderous academic discussion concerned with whether the osteoclast destroys healthy bone makes up a large share of the literature on this cell. Pommer [8] contended hotly in the affirmative, but numerous more recent workers are agreed that while the osteoclast may play an unimportant rôle in bone resorption, its chief function is that of an ordinary phagocyte.[9] It is found in tissues which produce fibroblasts and among those cells which in themselves are phagocytic. It seems logical to assume that osteoclasts represent a fusion, the fusion itself being of a phagocytic nature, of these cells.

The giant cell in the tumor of that name is the one which concerns us here. If it occurred only in giant cell tumors, we should admit the justification of the contention that the osteoclasts may become endowed with neoplastic properties to produce the tumor which writers with this belief term osteoclastoma, though even so it explains only one type of cell in the tumor and the prognostically unimportant one at that. But the giant cells of giant cell tumor, or cells morphologically indistinguishable, are found in a number of lesions with as many different causes. It is true that the giant cells of some giant cell tumors have certain features which set them apart from the osteoclast and the latter may often show characteristics which enable one to distinguish it from the giant cells of the brown tumor of hyperparathyroidism and unicameral cyst, but close scrutiny of a number of these lesions convinces me that the cells are often identical. If we begin with the premise that osteoclasts and the giant cells of giant cell tumor of bone, giant cell tumor of tendons, osteogenic sarcoma, fibrous dysplasia, hyperparathyroidism, unicameral cyst, osteoid osteoma, eosinophilic granuloma and allied reticulo-endothelioses, nonosteogenic fibroma, and ossifying fibroma are generically and functionally related, then the nature and differential diagnosis of benign giant cell tumor of bone become considerably simplified because without its giant cells the giant cell tumor of bone becomes simply a benign or malignant tumor of fibrous connective tissue—a fibroma, or a fibrosarcoma. I believe that such is the case and that all or most of the peculiarities of the giant cell tumor of bone can be logically explained on this premise.

In order better to comprehend the cytologic makeup of giant cell tumor and the conditions which produce similar microscopic pictures, a clinical, roentgenographic, and microscopic study of 115 primary lesions of bone was undertaken. The selected lesions were all non-inflammatory, with the possible exception of the reticulo-endothelioses.

These were included because of the difficulty which may arise in differ-entiating eosinophilic granuloma and giant cell tumor of bone. The incidence of the various lesions considered is shown below:

Giant cell tumor of bone	4
Giant cell tumor of tendon	12
Fibrous dysplasia	7
Hyperparathyroidism (von Recklinghausen's disease)	1
Unicameral cyst	8
Paget's disease	7
Osteoid osteoma	3
Osteogenic sarcoma	9
Enchondroma	4
Osteochondroma	17
Chondrosarcoma	12
Periosteal fibrosarcoma	6
Osteogenesis imperfecta	3
Ewing's tumor	9
Plasma cell myeloma	4
Myeloid myeloma	3
Reticulo-endothelioses	5
Primary epithelial cell tumor of bone (adamantinoma, tibia)	1
· Total	115

Giant Cell Tumors of Bone

The Giant Cells. The osteoclast was once believed to be the sole agent which brought about bone resolution. We now know that de-mineralization of bone is a chemical reaction depending upon a number of factors, chief of which are probably the blood supply and the reaction of the fluids which bathe the part affected. After deminerali-zation the soft fibrous matrix disintegrates because it is not living fibrous tissue but merely the collagenous product of fibrous cells. As elsewhere in the body, this tissue débris is cleared by cells the purpose of which is phagocytosis. In the brain, phagocytosis is accomplished by the gitter cells, modified microglia; in the soft tissues, it is accom-plished by macrophages and microphages which are derived from the reticulum either directly or by modification of fibroblasts. In bone, this important work is carried out by cells which in the embryo apparently arise directly from the mesenchyme and in postnatal life from the reticulum.[10,11] Just as in soft tissues in which the macrophages may be formed from abnormally proliferating fibroblasts, as in the tubercle or the foreign body reaction, so in bone they may be formed from fibroblastic cells which are exposed to an abnormal growth stimulus. Osteoclasts in abnormal numbers are seldom if ever found in healthy bone; they appear only after the lacunar cells are dead and bone has begun to disintegrate. Osteoclasis is usually a normal physiologic

process: in the embryo it clears the way for normal bone growth, in postnatal life it assists in the normal growth and maintenance of bone at a much slower pace. In abnormal situations, osteoclasts are found in large numbers most often in the presence of hemorrhage. Hemorrhage and its attendant change in the pH of the fluids and perhaps the extent of the blood supply cause bone resolution. That the osteoclast, itself an adult, functioning end-product, should acquire the proliferative characteristics of neoplasia is inconceivable. Giant cell tumors, no matter how malignant, rarely if ever show giant cells undergoing mitotic division. The giant cells may be the product of fused fibroblastic tumor cells or perhaps of incompletely separated littoral cells, but there is no evidence to show that they are self-propagating. In giant cell tumors any unusual features of their structure may be accounted for by their peculiar genesis, *i.e.*, from tumor cells. Their presence is the response to bone destruction caused by the tumor cells proper.

The Fibrous Matrix. The fibrous matrix of giant cell tumors is composed of cells of fibroblastic origin no more unusual than those of other fibrous tumors. It is these cells, however, as Jaffe[2] has so rightfully insisted, that determine the behavior of the tumor. A histologic diagnosis of giant cell tumor of bone must not be made unless the cells of the fibrous matrix are neoplastic in character. It is disregard of this very point which has led to such wild confusion in the descriptions of this tumor in the literature. The reason that many people believe that giant cell tumor is merely a response to trauma is because they have mistaken reparative cells for neoplastic fibrous cells. Their preoccupation with the giant cell has led them to consider two lesions, of dissimilar nature and prognosis, to be the same because one feature —the giant cells—was the same. In a like manner the behavior of the tumor can be predicted only by a consideration of the cells of the fibrous matrix. The giant cells give little or no clue as to whether the tumor will recur or metastasize, despite the advice from numerous quarters that the number of nuclei is inversely proportional to the degree of malignancy of the tumor.

The presence of foam cells is variable but they rarely make up any considerable part of the tumor. I have seen one giant cell tumor of a rib and another of a femur, however, in which some sections showed massive areas of these cells. I believe that the giant cell itself is made up of an agglomeration of matrix cells whereas the foam cell is elaborated directly from the reticulum. The hemorrhage and invaded marrow provide considerable amounts of lipid, and since these cells are phagocytic their cytoplasm soon becomes engorged. The presence of

foam cells does not distinguish a giant cell tumor of tendon from one of bone.

I am not implying that the histologic diagnosis of giant cell tumor of bone is an easy one. The most experienced pathologists may mistake an unusual reparative fibrous hyperplasia for tumor. Until this distinction can be made, one must be guarded about a diagnosis of giant cell tumor. Because of the inaccessibility of the lesion, insufficient material is often submitted by the surgeon, or the tumor is "uncorked" by removing a piece of the overlying cortex and the cork rather than the lesion itself is sent in for cytologic study. Even when adequate tissue is submitted, the pathologist should not hesitate to study the clinical history and the roentgenograms before venturing a diagnosis.

It is unquestionably true, as Jaffe[2] has pointed out, that giant cell tumors of bone rarely occur in young adults before the age of 20 years, and that they show a predilection for the epiphyses and the adjoining metaphyses of long bones. These characteristics are hard to explain, but an explanation is no more necessary to this discussion than is one for osteogenic sarcoma, unicameral cyst, osteoid osteoma, or the fibromas, each with its own site of predilection and age incidence. One is tempted to accept the theory that neoplasms of bone are most apt to occur at the site of growth where new cells are being formed, which, in the cylindrical bones, is at the epiphyseal line. Why the site of origin of the giant cell tumor is more often in the epiphysis than in the metaphysis where other medullary fibromas seem more prone to arise, is unknown to me unless it is because the more embryonic nature of the tissue of the epiphysis is more conducive to giant cell formation than other tissues, and growth therein establishes the peculiar nature of the tumor.

If the hypothesis that giant cell tumor of bone is a fibroma or fibrosarcoma with an unusual giant cell proliferation is correct, there might be some justification for suggesting a change of the name. Any such attempt, however, would certainly be futile. Furthermore, the tumor deserves a special designation because of its cytologic peculiarity. Perhaps the terms "giant cell fibroma" and "giant cell fibrosarcoma" might be more direct and explicit.

Differential Problems

Giant Cell Tumor of Tendons. The structure of ligaments and tendons bears many striking similarities to that of bone. The tissue is composed essentially of bundles of fibrils between which are caught spindle cells as lacunar cells are caught between lamellae of bone.

Tendon tissue is a product of mesenchyme just as is bone, although morphologically it more closely resembles ordinary fibrous connective tissue. Tumors may arise from the tendon itself or from its sheath. Those from the latter are usually classed with the endotheliomas as synoviomas or angiomas. Those arising from the tendons are collagen-producing and are therefore fibromas or, rarely, fibrosarcomas. Chronic inflammatory reaction is particularly prone to result in a productive lesion which may closely simulate a neoplasm. In the true tumors there are large numbers of giant cells and often a greater number of foam cells than in the giant cell tumor of bone.[12] Essentially, the course of this tumor is comparable to that of the giant cell tumor of bone with similar matrix components. The giant cell tumors of tendons grow slowly and, because they are more accessible, removal is usually not difficult. A few are malignant, extending along the course of the tendon and metastasizing late. Histologic differentiation from giant cell tumor of bone may be impossible, but unless the tumor has destroyed a considerable amount of bone, examination of roentgenograms should complete the diagnosis.

Osteogenic Sarcoma. During the enchondral formation of bone some of the mesenchymal matrix cells line up along the columns of cartilage to become osteoblasts. As the cartilage disappears the cells lay down osteoid tissue which later becomes calcified to form primitive bone. The osteoblasts from the inner layer of periosteum, or those from the medullary portion of the epiphyseal line may undergo neoplastic proliferation. These cells attempt to form bone, sometimes fairly successfully but more often so crudely that it may be difficult to recognize. Osteoblasts are derived from mesenchyme and they may fuse to form giant cells. Sometimes these cells are indistinguishable from those found in giant cell tumors, and since the tumor matrix is composed of collagen-producing spindle cells it may be virtually impossible to distinguish this tumor morphologically from a malignant giant cell tumor. The distinguishing feature is osteoid formation since the stem cell of the giant cell tumor normally produces fibrous connective tissue while the osteoblast produces bone. The problem becomes more complex when trying to differentiate the more "adult" type of giant cell tumor which may produce some bone, or the "young" type of osteogenic sarcoma in which bone formation is exceedingly crude or lacking. This circumstance suggests that the two lesions may merge one into the other depending upon the state of embryonic development of the stem cell. When the lesion is medullary, violates the cartilaginous end-plate, and is found in a patient over 20 years of age, the distinction had better

be postponed until more tissue can be studied or until the course of the lesion makes diagnosis possible. The only consolation to the pathologist, if not to the patient, is that if the distinction is difficult the lesion is surely malignant.

Fibrous Dysplasia. Fibrous dysplasia, particularly the monostotic variety, has been misdiagnosed roentgenographically as giant cell tumor of bone. This mistake should not be made after cytologic study. It is true that the matrix is composed of collagen-producing spindle cells, but, although they are young cells, they do not show the criteria for new growths.[13] An even more obvious difference is the production of primitive bone in fibrous dysplasia. The cells of giant cell tumor produce almost no bone whatsoever; where unquestionably produced by the tumor cells, it is a primitive, irregular type of osteoid tissue. Giant cells are often present but almost never in the numbers seen in giant cell tumor of bone. Incidentally, fibrous dysplasia, if both the monostotic and polyostotic varieties are included,[14] is by no means a rare disease. In my series of 115 primary lesions of bone there were 7 cases, while for the same period there were only 4 unquestionable cases of giant cell tumor.

Hyperparathyroidism (von Recklinghausen's Disease of Bone). Hyperparathyroidism is quite rare. In my series there was only one unquestionable case. Histopathologically, it is perhaps the lesion most easily confused with giant cell tumor of bone. Brown tumors are often quite numerous, interspersed among areas of cystic degeneration and osteoid proliferation. They are composed of masses of giant cells in a fibrous stroma. The cells of the latter should furnish the distinguishing feature. Yet if they are quite actively proliferating, I am not sure that I would care to make the distinction with finality. Some writers, including Jaffe,[15] believe that the giant cells of the two lesions are different. In the one case of my series I am unable to confirm this. distinction.

Solitary Unicameral Cyst. The solitary unicameral cyst is easily mistaken for giant cell tumor on roentgenologic evidence alone, although the epiphysis is seldom, if ever, involved. When the lesion is reasonably early, the age incidence is a helpful diagnostic feature.[16] There were 8 cases in my series, the oldest patient being 21 years of age. In the healing stage of a cyst there may be enough fibrous proliferation and giant cell formation to present a histologic picture not unlike that of giant cell tumor, but here again the giant cells are usually not numerous and the fibrous matrix lacks neoplastic characteristics.

Osteitis Deformans (Paget's Disease). The solitary focal type of

Paget's disease [17] in relatively young people might conceivably produce a roentgenogram which would simulate that of giant cell tumor. Histologically, the clear-cut mosaic pattern of the bone is not always present and there may be enough fibrous proliferation and giant cells in some areas to make a diagnosis of giant cell tumor seem reasonable. Usually the clinical history, the roentgenograms, and the cytologic structure, if considered together, will result in a correct diagnosis.

Osteoid Osteoma. Although there may be numerous giant cells in a fibrous matrix in the lesion which Jaffe [18] has named osteoid osteoma, the presence of calcified osteoid tissue usually makes the histologic diagnosis an easy one.

Reticulo-endotheliosis. Cases of reticulo-endotheliosis comprise a fascinating series, the complete pathology of which has not yet been established.[19] In my series there was one atypical case of Letterer-Siwe's disease, one well defined case of Hand-Schüller-Christian's disease, and 3 cases of eosinophilic granuloma. The last is the most likely to be mistaken for giant cell tumor of bone. When the eosinophilia is prominent the pathologist has little difficulty in arriving at a diagnosis, but sometimes eosinophils are few or absent [20] and giant cells may predominate in the section. When such is the case the evidence of inflammation must be relied upon to make the distinction.

Nonosteogenic Fibroma and Ossifying Fibroma. Nonosteogenic fibroma and ossifying fibroma derive their distinction as entities from their cytologic composition. In the former there are insufficient giant cells to warrant the diagnosis of giant cell tumor; in the latter the presence of considerable amounts of primitive bone serve as the distinguishing feature. One may question the advisability of complicating the classification of bone tumors by considering these tumors as separate entities.[21] If the giant cell tumor of bone were called giant cell fibroma, this objection might be overcome by considering all three lesions as variants of the same class. My experience with the fibromas is too limited to make any observation worth while.

CONCLUSIONS

I have presented the belief that the giant cells found in a number of bone lesions are identical or generically related cells produced for the purpose of phagocytosis. They should not be used for the purpose of distinguishing a giant cell tumor of bone. Fibrous matrix cells are found also in a number of bone lesions, both neoplastic and non-neoplastic. It is unfortunately true that many diagnoses of giant cell tumor of bone are based on these two criteria alone, a mistake which

makes the incidence abnormally high. This mistake is enhanced by the acceptance of roentgenologic characteristics which are common to a number of entirely different lesions.

The histologic diagnosis of giant cell tumor of bone should not be made unless the fibrous matrix cells are definitely neoplastic in character. Whether this tumor should be considered an entity apart from the other fibromas of bone is a question I am unable to answer. There seems to be some justification for this distinction in the clinical characters of the lesions. If they were called giant cell fibroma, non-osteogenic fibroma, and ossifying fibroma, the terminology would be self-explanatory and the classification would be simplified.

REFERENCES

1. Geschickter, C. F., and Copeland, M. M. Tumors of Bone. The American Journal of Cancer, New York, 1936, rev. ed., 832 pp.

2. Jaffe, H. L., Lichtenstein, L., and Portis, R. B. Giant cell tumor of bone. Its pathologic appearance, grading, supposed variants and treatment. *Arch. Path.*, 1940, 30, 993–1031.

3. Chatterton, C. C., and Flagstad, A. E. Peculiar behavior of giant-cell tumors. *J. Bone & Joint Surg.*, 1927, 9, 111–118.

4. Aegerter, E. E., and Peale, A. R. Kaposi's sarcoma. A critical survey. *Arch. Path.*, 1942, 34, 413–422.

5. Maximow, A. A., and Bloom, W. A Textbook of Histology. W. B. Saunders Co., Philadelphia, 1942, ed. 4.

6. Mallory, F. B. Giant cell sarcoma. *J. M. Research*, 1911, 24, 463–467.

7. von Köllicker, A. Die normale Resorption des Knochengewebes und ihre Bedeutung für die Entstehung der typischen Knochenformen. F. C. W. Vogel, Leipzig, 1873.

8. Pommer, G. Ueber die Ostoklastentheorie. *Virchows Arch. f. path. Anat.*, 1883, 92, 296–363.

9. Lewis, F. T. A Textbook of Histology. P. Blakiston's Son & Co., Philadelphia, 1944, ed. 6.

10. Jackson, C. M. Zur Histologie und Histogenese des Knochenmarkes. *Arch. f. Anat. u. Physiol., anat. Abteil.*, 1904, 33–68.

11. Arey, L. B. The origin, growth and fate of osteoclasts and their relation to bone resorption. *Am. J. Anat.*, 1919–20, 26, 315–345.

12. Galloway, J. D. B., Broders, A. C., and Ghormley, R. K. Xanthoma of tendon sheaths and synovial membranes. A clinical and pathologic study. *Arch. Surg.*, 1940, 40, 485–538.

13. Lichtenstein, L. Polyostotic fibrous dysplasia. *Arch. Surg.*, 1938, 36, 874–898.

14. Stauffer, H. M., Arbuckle, R. K., and Aegerter, E. E. Polyostotic fibrous dysplasia with cutaneous pigmentation and congenital arteriovenous aneurysms. A case report. *J. Bone & Joint Surg.*, 1941, 23, 323–334.

15. Jaffe, H. L. Hyperparathyroidism (Recklinghausen's disease of bone). *Arch. Path.*, 1933, 16, 63–112.

16. Jaffe, H. L., and Lichtenstein, L. Solitary unicameral bone cyst with emphasis on the roentgen picture, the pathologic appearance and the pathogenesis. *Arch. Surg.*, 1942, 44, 1004–1025.

17. Jaffe, H. L. Paget's disease of bone. *Arch. Path.*, 1933, 15, 83–131.

18. Jaffe, H. L. "Osteoid osteoma." A benign osteoblastic tumor composed of osteoid and atypical bone. *Arch. Surg.*, 1935, 31, 709–728.

19. Green, W. T., and Farber, S. "Eosinophilic or solitary granuloma" of bone. *J. Bone & Joint Surg.*, 1942, 24, 499–526.

20. Jaffe, H. L., and Lichtenstein, L. Eosinophilic granuloma of bone. *Arch. Path.*, 1944, 37, 99–118.

21. Jaffe, H. L., and Lichtenstein, L. Non-osteogenic fibroma of bone. *Am. J. Path.*, 1942, 18, 205–221.

[*Illustrations follow*]

DESCRIPTION OF PLATES

Figure 1 was taken from a giant cell tumor of bone. Any of the other lesions illustrated might have been misdiagnosed giant cell tumor of bone on a cytologic basis alone, if the part examined were restricted to the areas containing giant cells in a fibrous matrix. Indeed, several of the lesions were so diagnosed by reputable pathologists. The structure of the giant cells varies considerably even within the limits of the same lesion and similar variations are found in a variety of lesions of different types. All of the sections illustrated were stained with hematoxylin and eosin and photographed at a magnification of 300.

PLATE 47

FIG. 1. Male, 22 years of age. Giant cell tumor of bone: destruction of rib. Surgical resection brought about complete healing. There was no recurrence 8 years later. The giant cells varied considerably in size, shape, and number of nuclei. Some resembled those in Figure 4, an instance of hyperparathyroidism.

FIG. 2. Female, 43 years old. Giant cell tumor of tendon: tumor of tendons of palm. Surgical resection brought about complete healing. There was no recurrence 6 years later.

FIG. 3. Male, 11 years of age. Fibrous dysplasia: demineralization and deformity of the shaft of the left tibia. Treatment was by curettage and introduction of bone chips. There was no advancement of the process after 7 years.

FIG. 4. Female, 25 years old. Hyperparathyroidism: focal demineralization of femur, humerus, skull, vertebrae, and phalanges and moderate generalized demineralization, of 3 years' duration. Serum calcium was 11.9 mg. per cent. Calculi were present in both kidneys. A parathyroid adenoma, 1.5 cm. in diameter, was removed. Death occurred on the second postoperative day.

1
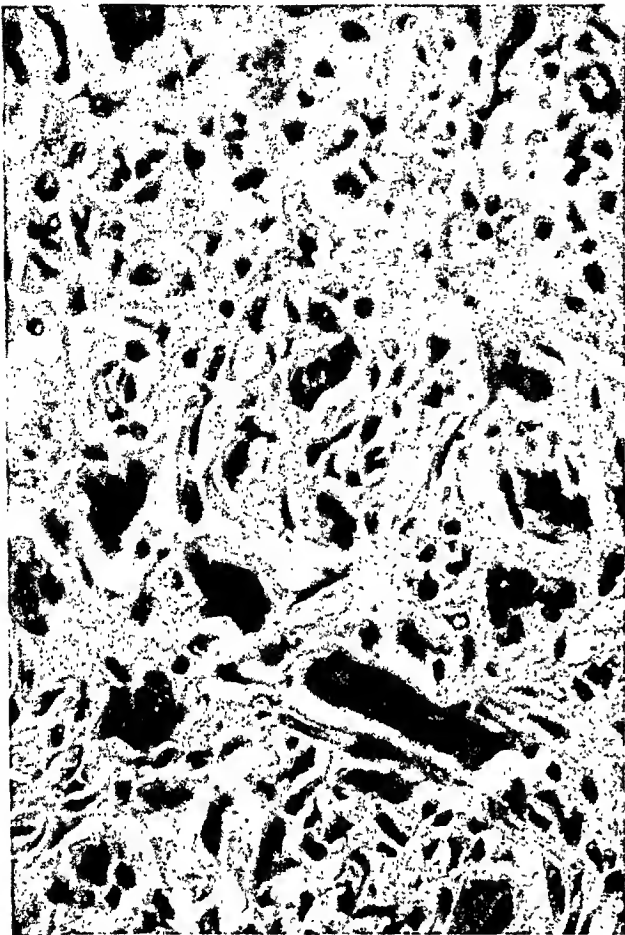
2
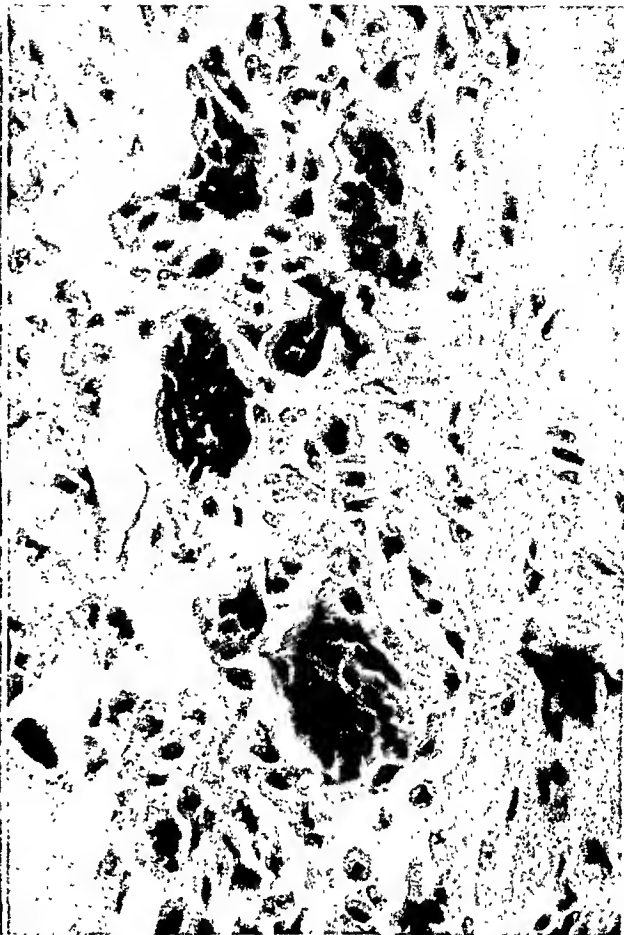
3
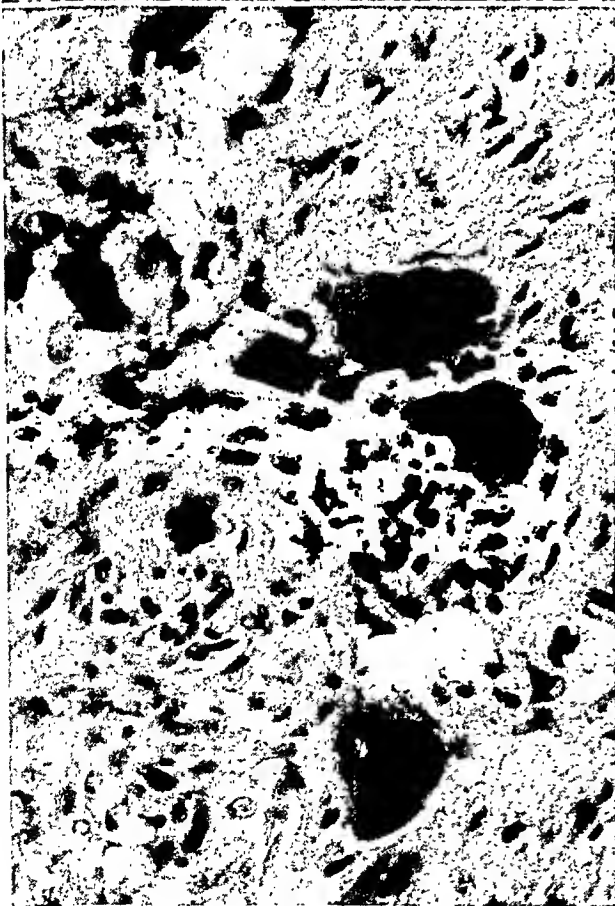
4
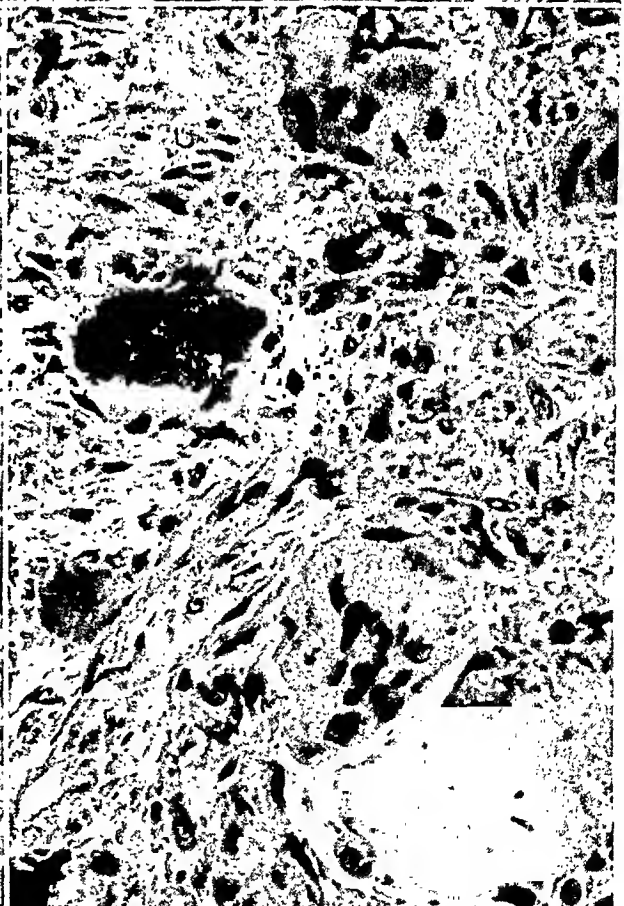
Aegerter

Giant Cell Tumor of Bone

PLATE 48

FIG. 5. Female, 6 years old. Unicameral cyst: focal demineralization in the metaphysis of the left tibia. Curettage effected complete healing. There was no recurrence 7 years later.

FIG. 6. Male, 42 years of age. Paget's disease: thickening and mottled demineralization of the skull and extremities, fracture of femur and tibia. Specimen taken for biopsy revealed the typical mosaic of Paget's disease. Subsequent course was not recorded.

FIG. 7. Male, 25 years old. Osteoid osteoma: focal demineralization of the proximal radius, of 2 years' duration. The head of the radius was excised. There was no recurrence after 7 years.

FIG. 8. Female, 29 years of age. Eosinophilic granuloma: focal demineralization of the 7th and 8th ribs and the 7th thoracic vertebra. The 7th rib was resected; the other lesions regressed after 2 years.

5
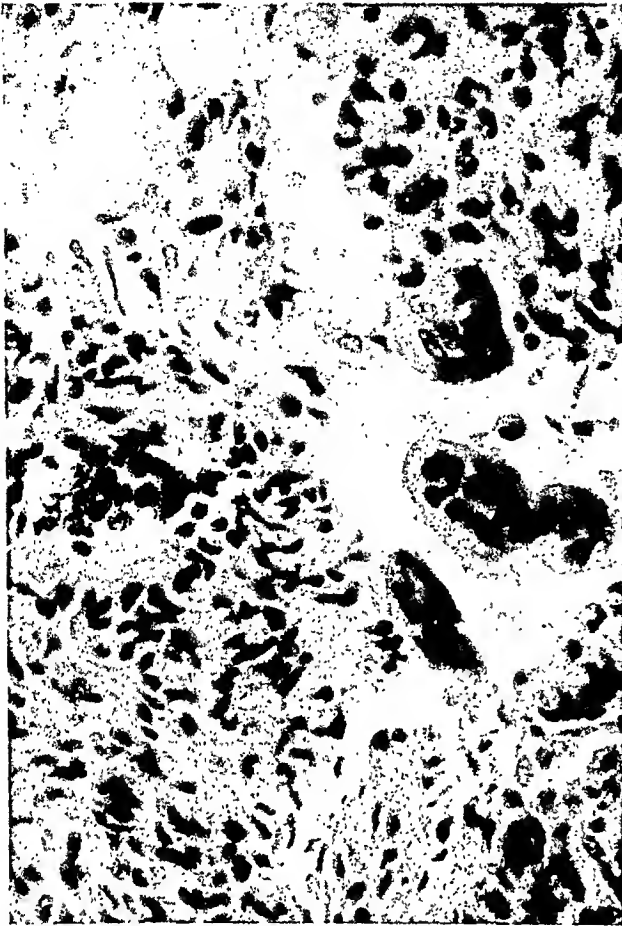
6
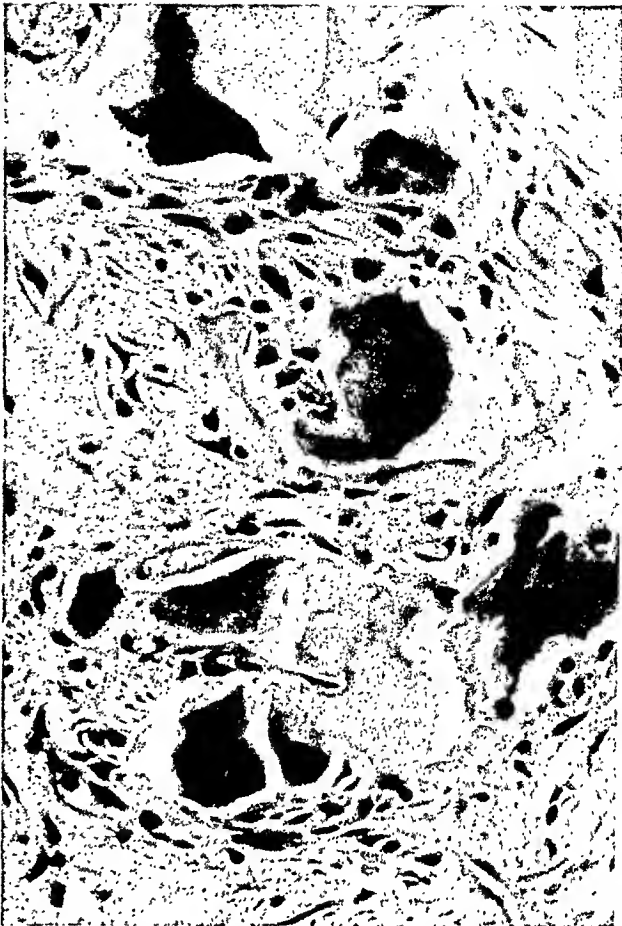
7
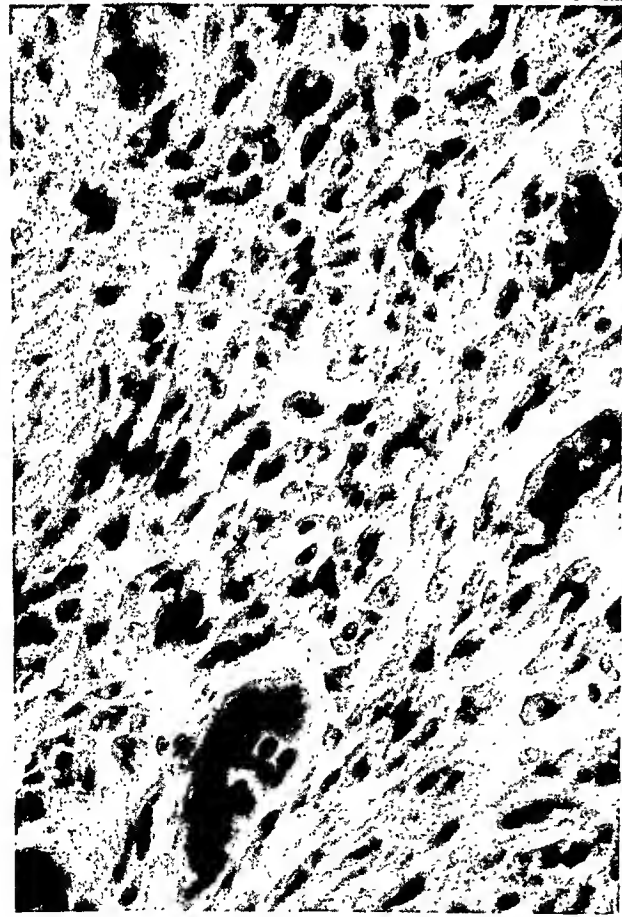
8
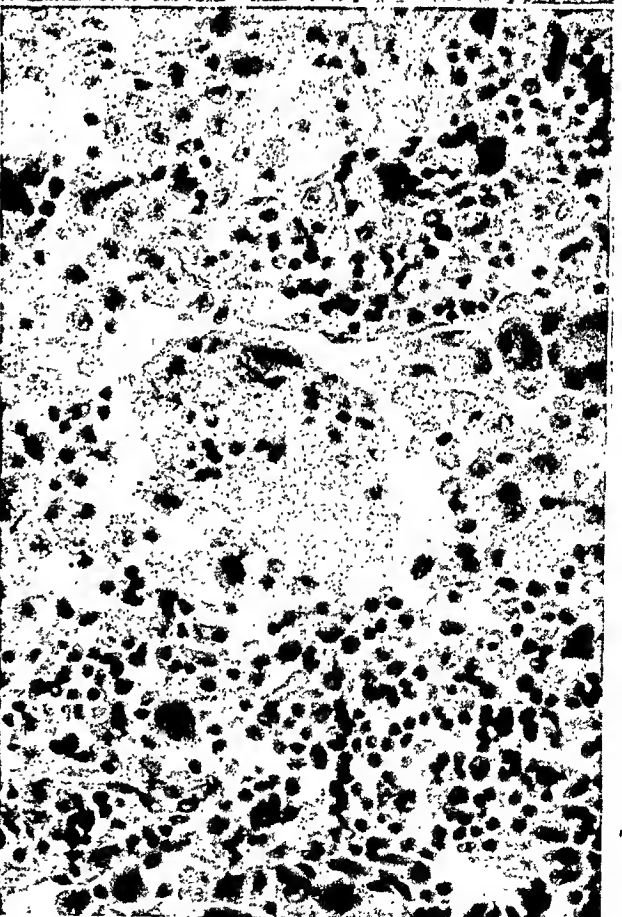
Aegerter   Giant Cell Tumor of Bone

RESIDUAL TISSUE CHANGES IN MALE DOGS FOLLOWING CESSATION OF ORALLY ADMINISTERED STILBESTROL *

R. M. Mulligan, M.D., and D. L. Becker, M.D.

(From the Department of Pathology, University of Colorado, School of Medicine, Denver 7, Colo.)

The clinical and pathologic changes in male dogs which had received oral stilbestrol were described in a previous paper.[1] When the third dog presented in that experiment was autopsied several months after the cessation of medication, he showed certain anatomic findings which were thought to represent the residual effects of the stilbestrol. In order to investigate these organic lesions further, four other male mongrel dogs were fed stilbestrol. One succumbed early in the experiment. The other three were examined anatomically at varying lengths of time after withdrawal of the drug. The significant clinical, hematologic, and anatomic abnormalities observed in these five animals form the basis for this report.

Protocols of Experiments

Dog S-E was a long-haired, springer-spaniel mongrel about 2 years old and weighing 13.6 kg. Following a rib resection for marrow studies on January 20, 1945, he received 770 mg. of stilbestrol in 5 doses of 10 mg. and 48 doses of 15 mg. in the 60 days inclusive of January 29 through March 29, 1945. This was an average dose of 56.6 mg. per kg., or 0.94 mg. per kg. per day. On the 49th day he was inappetent, listless, and thin. By the 58th day, he had lost much weight and appeared toxic. The floor of his mouth was swollen and hemorrhagic and he began to pass bloody, semiliquid stools. He failed rapidly, lapsed into coma, and died on the 61st day, March 30, when he weighed about 9 kg. The wound of the skin for rib resection was broken down, hemorrhagic, and crusted. The hair at the operative site was not fully regenerated. The significant gross findings included severe loss of body fat; hemorrhagic right auricle; pulmonary hypostasis; small flabby spleen; widespread ecchymoses in the gastrointestinal mucosa; flabby kidneys with swollen bright yellow cortices and pale pink pyramids; fairly plentiful, semifluid, dark red costal and vertebral bone marrow; a small, soft prostate, and testes about one-third normal size.

Dog S-F was a moderately long-haired, St. Bernard-pointer mongrel, about 2 years old and weighing 15 kg. Following removal of marrow from a rib for biopsy on February 3, 1945, he received 1910 mg. of stilbestrol in one dose of 20 mg., and 126 doses of 15 mg. in 180 days inclusive of February 6 through August 4. This was an average dose of 127.3 mg. per kg., or 0.71 mg. per kg. per day. On the 77th day, when he had received 935 mg. of the drug, his appetite decreased and he began to lose weight and vigor. The stilbestrol was stopped for 21 days, during which time his general condition improved toward normal. On the 192nd day, he weighed 16.8 kg. and showed enlarged breasts, moderate swelling of the penile sheath, testes about three-fourths normal size, prostate about two-thirds normal size, general thinning of the hair, and loss of hair from the ventrum of the abdomen, the penile sheath, the perineum, and adjacent thighs. Serum agglutination for *Leptospira canicola* was positive in dilutions of 1:10 to 1:100 and for *Leptospira*

---

icterohaemorrhagiae in dilutions of 1:10 to 1:1000. On the 220th day, September 13, 40 days after cessation of stilbestrol, the breasts, testes, and penile sheath were within normal limits and the hair was partly regenerated in areas from which it had been lost. He also displayed increased fullness in the ventral abdominal wall, giving the impression of a female type of body configuration, also seen in dogs S-C and S-A to be described. The significant gross findings at autopsy of dog S-F included short, pale yellow streaks at the corticomedullary junction of the kidneys and a slightly contracted prostate.

Dog S-C was a short-haired, greyhound mongrel about 2 years old and weighing 19 kg. Following removal of marrow from a rib for biopsy on December 23, 1944, he received 3520 mg. of stilbestrol in 176 doses of 20 mg. in 208 days inclusive of January 9 through August 4, 1945. This was an average dose of 185.3 mg. per kg., or 0.89 mg. per kg. per day. By the 116th day, the hair had partly regenerated at the site of the rib resection and was much lighter brown than the adjacent hair. On the 288th day, hair loss involved most of the ventral abdominal wall, the perineum, adjacent thighs, and the penile sheath. The breasts were enlarged, the prepuce swollen, and the prostate and testes shrunken. He weighed 20.5 kg. On the 332nd day, December 6, 124 days after cessation of the stilbestrol, the hair, prepuce, penile sheath, and testes were within normal limits, but the breasts were slightly enlarged and the prostate was somewhat shrunken. No other significant gross changes were observed at autopsy.

Dog S-A was a short-haired, terrier-bull mongrel, about 3 years old and weighing 11.5 kg. Following removal of marrow from a rib for biopsy on December 29, 1944, he received 1770 mg. of stilbestrol in 177 doses of 10 mg. in 208 days inclusive of January 19 through August 4, 1945. This was an average dose of 153.9 mg. per kg., or 0.74 mg. per kg. per day. By the 219th day, when he weighed 12.3 kg., the breasts were enlarged, the penile sheath was greatly swollen and hairless, the testes were shrunken, the prostate was enlarged and moderately hard, and the ventral abdominal wall and perineum were devoid of hair. On the 381st day, January 24, 1946, 173 days after cessation of stilbestrol, the hair had been regenerated and the prepuce, penile sheath, and testes were within normal limits. The breasts were slightly, and the prostate moderately, enlarged. The significant autopsy findings included a hypertrophied urinary bladder and an enlarged prostate with excavations in the dorsal parts of the lateral lobes filled with cheesy material.

Dog 52, the third dog described previously,[1] was a long-haired, water-spaniel mongrel about 1½ years old and weighing 8 kg. Following removal of marrow from a rib for biopsy on March 17, 1942, he received 1850 mg. of stilbestrol in 280 days inclusive of April 20, 1942, through January 25, 1943, and 500 mg. of the same compound subfascially on the 280th day. This was an average oral dose of 231 mg. per kg., or 0.83 mg. per kg. per day. On the 294th day, he showed enlarged breasts, greatly depressed libido, a swollen penile sheath, shrunken testes, and extensive hair loss. On the 583rd day, the clinical appearance of this dog was normal and did not appreciably change by the 791st day, May 16, 1944, 511 days after cessation of the stilbestrol, except as related to the site of implantation of methylcholanthrene as reported.[2] His final weight was 10.5 kg.

The brains of all five dogs were grossly normal.

METHODS AND MICROSCOPIC OBSERVATIONS

The tissues obtained at autopsy were fixed in Zenker's fluid, imbedded in paraffin, cut at 6 μ, and stained with hematoxylin and eosin, except that the right adrenal glands of the dogs were fixed in 4 per cent formaldehyde and sections of them were frozen with carbon dioxide gas, cut at 15 μ, and stained with sudan IV. The smears of

costal marrow were stained by the May-Grünwald-Giemsa method following preparation by the serum technic. The pituitary glands cut in sagittal section were stained by Mallory's connective tissue stain. The tissues examined microscopically included the thymus, thyroid and parathyroid glands, the heart, lungs, spleen, esophagus, stomach, intestines, liver, gallbladder, pancreas, adrenal glands, kidneys, bladder, prostate, vasa efferentia, testes, epididymides, breasts, lymph nodes, bone marrow, skin, skeletal muscle, and pituitary gland.

Adrenal Glands

In dog S-E, the adrenal cortex (Fig. 1) was moderately increased in width and had a smooth border applied evenly to the regular capsule. The shrunken cells of the zona glomerulosa had pyknotic nuclei and acidophilic cytoplasm containing few to many small granules of lipid. The nuclei of the small fascicular cells were condensed to varying degrees and the acidophilic cytoplasm contained little or no fat. Many fascicular cells, especially those along the inner edge of the fascicular zone, had eccentric pyknotic nuclei, were enlarged and rounded, and inclosed large globules of fat. An increase of the cells in the reticular zone resulted in an absolute widening of this layer and accounted for the greater thickness of the cortex. The cells of the zona reticularis resembled those in the fascicular zone, but were more flattened and contained decidedly fewer granules and globules of fat.

In dog S-F, the adrenal cortex (Fig. 2) was quite narrowed and scalloped in irregular folds beneath the capsule, which dipped down between stretches of undulated thinned cortex. The moderately shrunken glomerulosal cells showed slightly condensed nuclei, increased cytoplasmic acidophilia, and irregular decrease of fat. The fascicular layer was greatly thinned by the decrease in size and lipid content of the constituent cells, which contained nuclei condensed to varying degrees, even to pronounced pyknosis. The reticular layer was moderately thickened and the reticular cells contained little or no fat.

In dog S-C, the adrenal cortex (Fig. 3) was narrowed and peripherally scalloped. The glomerulosal cells were crowded together, showed increased cytoplasmic acidophilia, contained varying amounts of fine fat droplets, and had fairly well preserved nuclei. Especially in the outer third of the fascicular zone, the cells were smaller, severely depleted of lipid, and marked by condensed nuclei, also seen in scattered cells in the inner two-thirds. Most of the zona reticularis lacked lipid.

In dog S-A, the adrenal cortex (Fig. 4) had fairly uniform thickness and a smooth outer border. The glomerulosal cells were smaller, bunched together, and marked by fine fat granules. The cells of the

outer margin of the zona fasciculata were shrunken and depleted of
lipid. The cells in the remainder of this zone were filled with fine and
medium fat granules, save for a few scattered cells with condensed
nuclei and cytoplasm ballooned by large fat globules. The unevenly
widened zona reticularis consisted of partly flattened cells containing
few fat granules.

The adrenal cortex of dog 52 was not appreciably different from
that of dog S-A. The adrenal gland (Fig. 5) of a control dog had
an outer cortical border, well rounded beneath the regular, smoothly
applied capsule. The intact cells of the glomerulosal and fascicular
layers were entirely filled with fine fat droplets. The width of the zones
was evenly developed, especially that of the fascicular zone. The quite
narrow reticular zone contained small numbers of partly degenerated
cells swollen with large globules of lipid.

Kidneys

In the kidneys of dog S-E, the stroma, especially of the cortex,
contained numerous infiltrations of lymphocytes, plasma cells, and
monocytes which surrounded damaged or atrophic tubules. Several
groups of atrophic convoluted tubules were imbedded in increased
fibrous connective tissue infiltrated by similar inflammatory cells. With
both Levaditi and Dieterle stains many spirochetes with the structure
of Leptospira were present in these infiltrations, both free and within
macrophages, in the form of intact organisms and disintegrated gran-
ules. Masses of spirochetes (Fig. 6) and many granules were present
in the lumen and intermingled with the lining cells of several fairly
well preserved convoluted tubules.

In the kidneys of dog S-F, radial, cortical, interstitial infiltrations of
lymphocytes and a few pigmented macrophages surrounded atrophic
tubules and glomeruli with fibrotic capsules. In both cortex and
medulla irregular, radial areas of increased fibrous connective tissue
were present. A few glomeruli were obliterated by fibrous connective
tissue. Groups of dilated collecting tubules were filled with hyaline
casts. The Dieterle stain failed to reveal spirochetes either in the
stromal infiltrations or within the tubules.

The kidneys of dogs S-C, S-A, and 52 revealed no significant histo-
logic abnormalities.

Prostate

In dog S-E, the utricle, the prostatic urethra, and the ducts and
acini lateral and dorsal to the urethra showed stratified squamous
epithelial metaplasia. The remaining atrophic ducts and acini were

lined by simple squamous or cuboidal epithelium. The stroma around the utricle was moderately infiltrated by lymphocytes and plasma cells.

In dog S-F, the prostatic urethra showed squamous epithelial metaplasia. The transitional epithelium of the ducts and the columnar epithelium of the acini were flattened and the lining cells were shrunken. A few peripheral acini were lined by undulatory columnar epithelium. A few small foci of lymphocytes marked the lamina propria of the urethra and the stroma. An occasional duct or acinus inclosed acidophilic coagulum and a few scattered segmented neutrophils.

In dog S-C, the prostatic urethra showed squamous epithelial metaplasia. The epithelium lining the ducts and acini was flattened. The small acini were lined by a low-cuboidal epithelium with prominent reserve cells. The shrunken peripheral acini were lined by a mildly undulatory low-columnar epithelium. The stroma contained a few small foci of lymphocytes.

In dog S-A, the epithelium over the verumontanum was stratified squamous in type. The epithelium of the ducts was flattened or involved by focal squamous metaplasia. In the dorsal parts of the lateral lobes, the ducts and some acini were greatly dilated; contained masses of segmented neutrophils, shed epithelial cells, and acidophilic fluid; and were lined by epithelium studded with abscesses. The frequently dilated acini were lined by epithelium which was atrophic or transformed to a stratified squamous type. A mildly undulatory columnar epithelium lined a few peripheral acini. The stroma was extensively infiltrated by lymphocytes and plasma cells, especially around the enlarged ducts in the dorsal parts of the lateral lobes.

In dog 52, the prostatic urethra was without change. The main parts of the ducts, especially those to the dorsal parts of the lateral lobes, were dilated and lined by transitional epithelium focally metaplastic to stratified squamous epithelium. Many shrunken acini were lined by flattened low-columnar epithelium or by a squamous epithelium with two or three layers. Nodules of lymphocytes were abundant in the stroma.

Testes

In the testes of dog S-E, the tubules were small. The seminal epithelium consisted largely of spermatogonia, scattered sustentacular cells, and a few primary spermatocytes. The stroma was relatively increased.

In the testes of dog S-F, the tubules were slightly decreased in size. The seminal epithelium was developed through the secondary sperma-

tocyte stage in most tubules, through the primary spermatocyte stage in some, and through the spermatid stage in a few. No well formed spermia were identified. The stroma was not remarkable.

In dogs S-C, S-A, and 52, the tubules of the testes were of normal size, the seminal epithelium was well preserved, and spermiogenesis was active. The stroma was delicate and inconspicuous.

Penile Sheath

In dog S-E, the epithelium of the penile sheath was hypercornified, thickened, moderately acanthotic, and free of infiltrated inflammatory cells. The basal layer contained mitotic figures. The small, fairly discrete lymphatic nodules in the lamina propria were occasionally marked by small deposits of hyalin.

In dog S-F, the epithelium of the penile sheath was hypercornified, moderately acanthotic, and thickened. Sloughed epithelial cells were present in the lumen. The lamina propria contained only a few lymphocytes.

In dogs S-C, S-A, and 52, the epithelium of the penile sheaths was slightly cornified, showed well marked acanthosis, was widely but lightly infiltrated by segmented neutrophils, and had discrete rete pegs. In the lamina propria, infiltrated by lymphocytes and plasma cells, clearly delineated lymphatic nodules bulged beneath the overlying epithelium. This histologic picture was consistent with that normally seen.

Breasts

In dog S-E, the mammary ducts were greatly increased in size and number. Many acini, not present in the normal male canine breast had proliferated. The dilated ducts were lined by pseudostratified tall-columnar epithelium thrown into papillary folds and contained acidophilic coagulum and degenerated epithelial cells. The acini were numerous and lined by papillary tall-columnar epithelium.

In dog S-F, the mammary ducts were increased in number, dilated, and lined by epithelium varying from transitional to low-columnar in type. The acini were tremendously increased in number and lined by low-columnar or cuboidal epithelium, the cells of which had either well preserved cytoplasm and nuclei, or swollen, rounded, hyaline cytoplasm and pyknotic, often eccentric, nuclei.

In dog S-C, the mammary ducts were moderately increased in number, size, and length, showed narrow or sometimes slightly widened lumina, and were lined by transitional epithelium. In small lobules at the ends of the ducts were proliferated acini lined by cu-

boidal epithelial cells containing condensed nuclei. The acinar lumina were slit-like or filled with solid clusters of epithelial cells.

In dog S-A, the mammary ducts were increased in size and number, lined by transitional or two-layered columnar epithelium, and moderately dilated. The acini, greatly increased in number and complexity, were lined by well developed low-columnar epithelium. Scattered acinar cells had swollen, rounded, hyaline cytoplasm and pyknotic, eccentric nuclei.

In dog 52, only a few small ducts near the nipple were found, an appearance consistent with that in the normal male canine breast.

Blood and Bone Marrow

The values for the hemoglobin and erythrocytes of the peripheral blood and for the differential count of 500 nucleated cells of the rib marrow are summarized in Tables I and II.

Thyroid and Pituitary Glands

Qualitative analyses of the thyroid and pituitary glands of the five experimental animals are given in Tables III and IV.

COMMENT

The changes seen in the adrenal glands at the height of the action of stilbestrol in dog S-E and in other dogs [1] and the gradual recuperation of the cortex of these structures as observed in dogs S-F, S-C, S-A, and 52 suggest that the adrenal cortex recovers, but not completely, from the injury inflicted on it by stilbestrol. By interpolation,

TABLE I

Values for Erythrocytes and Hemoglobin in the Peripheral Blood

Dog	Date	Days	Period	Red blood cells	Hemoglobin
				(millions)	(gm.)
S-E	1/20/45		C	7.69	14.0
S-F	2/3/45		C	7.15	14.5
	5/1/45	84	E	5.98	11.0
	8/17/45	192	M	4.91	10.1
	9/13/45	220	T	6.38	11.4
S-C	12/23/44		C	9.58	17.4
	5/5/45	132	E	9.96	22.0
	8/22/45	228	M	7.33	14.2
	12/6/45	332	T	8.86	18.4
S-A	12/29/44		C	7.93	14.3
	5/22/45	149	E	7.00	18.2
	8/13/45	219	M	6.58	12.5
52	3/17/42		C	5.99	14.2
	9/29/42	158	E	5.91	13.8
	1/25/43	280	M	5.07	11.2
	5/16/44	791	T	6.81	15.2

C = control period; E = early average experimental values;
M = values at time of maximum oral stilbestrol; T = terminal values.

TABLE II

Differential Counts Based on 500 Nucleated Cells of Rib Marrow at the Start of the Experiments and at Autopsy at Their Termination

Dog	S-E		S-F		S-C		S-A		52	
Date	1/20/45	3/30/45*	2/3/45	9/13/45	12/23/44	12/6/45	12/20/44	1/24/46	3/17/42	5/16/44
Eosinophils	1.8	0	4.0	3.4	3.2	2.2	3.0	2.8	2.0	2.4
Neutrophils:										
Promyelocyte	2.6	0	2.2	1.4	1.4	2.6	0.8	1.6	1.8	1.2
Myelocyte	7.8	0	5.2	7.2	5.4	7.6	6.6	9.2	8.0	4.2
Metamyelocyte	14.8	rare	14.2	14.0	13.0	9.0	13.0	16.0	19.4	15.4
Band forms	28.2	rare	37.0	16.2	25.0	14.6	25.6	25.8	32.4	22.4
Segmented	3.8	rare	2.2	2.8	3.4	1.0	1.0	1.6	3.2	2.8
Pro-erythroblasts	0.8	0	0.2	0.2	0.0	0.4	0.0	0.8	0.6	0.2
Erythroblasts	3.4	rare	1.6	1.4	2.2	1.0	2.6	3.0	1.6	2.6
Normoblasts	29.2	rare	29.0	44.0	42.2	51.4	41.4	30.8	26.0	40.4
Lymphocytes	3.6	few	2.0	3.2	2.4	4.8	1.8	3.4	2.8	4.8
Miscellaneous and unidentified cells	4.0	†	2.4	6.2	1.8	5.4	4.2	5.0	2.2	3.6
Megakaryocytes	many	rare	many	many	many	many	many	few	many	many
Marrow cell/fat cell ratio	65/35	2/98	65/35	70/30		80/20	65/35	70/30	65/35	65/35
Myeloid/erythroid ratio	1.8		2.1	1.0	1.2	0.72	1.1	1.7	2.4	1.1

* Cells too few for differential count.
† Many hemosiderin macrophages.

a dynamic picture of the serial recovery of the adrenal cortex may be drawn. Apparently, stilbestrol injures cells of the germinal layer, or zona glomerulosa, so that their function is impaired as they migrate into the functional layer, or zona fasciculata. Because of this impaired function, the cells do not elaborate the amount of lipid normally seen in them. They are injured in such a way that they degenerate earlier into the ballooned cells filled with large globules of fat, ordinarily seen only in the normal zona reticularis, or degenerative layer. Because of this earlier degeneration, the zona reticularis is greatly widened, relatively devoid of lipid, and concerned only with the removal of degenerated cells from the zona fasciculata, but not with the disposal of their usually abundant remaining lipid. In the process of recovery, the glomerulosal and fascicular zones show gradual widening of the formerly narrowed cortex and regeneration of new functional cells from the germinal layer with an increasing complement of lipid, so that a condition approaching that of the normal gland is attained.

When the adrenal glands of dogs S-A and 52 were compared with normal glands, the peripheral fascicular cells with less lipid and widened reticular zone suggested that a residual effect of stilbestrol may be to cause a delayed start in the evolution of the fascicular cell from the glomerulosal cell, so that a functional cell with a much shorter effective life is produced and one which consequently degenerates earlier than the normal fascicular cell.

The kidney damage in dogs S-E and S-F was reflected clinically to a striking degree. Ordinarily, with protracted dosage, stilbestrol does not interfere with the general well-being of a male dog, as illustrated by dogs S-C, S-A, 52 and others,[1] when the total amount has

TABLE III

Comparison on a Percentage Basis of the Types of Follicles in the Thyroid Glands

Follicular types	Dog S-E	Dog S-F	Dog S-C	Dog S-A*	Dog 52†
Small, solid polyhedral cells, no colloid	0	15	5	5	5
Reduced size, cuboidal epithelium, little colloid	100‡	50	20	10	0
Normal size, low-cuboidal epithelium, much bright colloid	0	35	20	20	95
Normal size, high-cuboidal epithelium, colloid edges vacuolated	0	0	55	65	0

* Stroma marked by minute to large foci of lymphocytes.
† Several follicles partly or completely filled by intensely basophilic particles about 1 to 10 μ in diameter.
‡ Cells swollen and granular; nuclei pyknotic; colloid absent from most follicles; degenerated epithelial cells in some follicles.

reached only 770 mg. as in dog S-E, or 935 mg. as in dog S-F. Some cause connected with kidney damage must have been the underlying basis for the constitutional reaction in these two animals. Zondek and Sulman[3] studied the inactivation of estrone, of conjugated natural estrogens, and of stilbestrol in infantile rats. They found that stilbestrol is rendered inactive *in vivo* to a small extent as compared to estrone. These authors showed that the esters of natural estrogens were similar to stilbestrol in their absorption, but once taken into the circulation were split and metabolized much like estrone, which was excreted in only small amounts. In contrast, stilbestrol was inactivated in the organism to but a slight extent and much of the free compound was excreted, mainly in the urine. Other experiments showed that liver cells are responsible for detoxifying natural estrogens by both destruction and conjugation. On the other hand, stilbestrol is little affected by the action of liver cells, although, similar to natural estrogens, it is eliminated in small amount in the bile. References to these experiments have been given.[4] Although the livers of dogs S-E and S-F were normal, their damaged kidneys probably seri-

ously hindered the excretion of stilbestrol from the blood stream. Thus a much higher effective level probably was present in the circulation with a much smaller total dose than in dogs with normal kidneys, so that changes characteristic of large amounts of stilbestrol administered in a short period were produced in the bone marrow, testes, prostate, and thyroid and adrenal glands of dog S-E and untoward clinical symptoms developed in dog S-F.

The estrogenic changes seen in human beings with cirrhosis of the liver have been appreciated in this country in more recent years.[5, 6] The rôle of damaged kidneys in the heightening of these changes has been stressed but little or not at all. The observations in dogs S-E and S-F suggest that human patients with cirrhosis of the liver and chronic kidney disease might show more profound estrogenic effects than those

TABLE IV

Comparison on a Percentage Basis of the Cell Types in the Anterior Lobes of the Pituitary Glands

Cell types	Dog S-E	Dog S-F	Dog S-C	Dog S-A	Dog 52
Chromophobes	15	48	33	42	35
Acidophils	84	50	37	55	60
Basophils	1	2	30	3	5

with only hepatic cirrhosis. Possibly synthetic estrogens should be given cautiously to human patients with damaged kidneys, since the estrogenic effects known to be produced in human tissues might thereby be intensified. Fortunately, the profound effects of estrogens on canine bone marrow have not been proved to occur in man.

The evidence in dog S-E indicated that the animal was suffering from leptospirosis, probably of the canicola type. The only definite pathologic changes attributable to the disease were found in the kidneys. The kidneys of dog S-F did not show spirochetes, but were involved by clear-cut chronic interstitial nephritis, possibly a sequel of former active leptospirosis, indicated by positive serum agglutinins. Smith and Jensen [7] have been impressed by the presence of chronic interstitial nephritis in several dogs following recovery from proved leptospirosis and believe that this type of canine nephritis may be a result of sensitization of the renal parenchyma to Leptospira, which cannot be demonstrated once the animal has recovered from active leptospirosis.

The return of the parenchyma of the prostate to a normal state following injury by stilbestrol was not complete in any of the dogs. The parts of the prostate most affected by apparently irreparable damage were the ducts and acini leading to the dorsal parts of the lateral lobes.

The testes of the animals surviving longest did not differ significantly from the normal, indicating the powers of regeneration of the canine seminal epithelium following injury by stilbestrol.

The changes in the penile sheath at the height of stilbestrol stimulation confirmed those previously described.[1] The recovery in three dogs from the inflicted damage was apparently complete.

Although the ducts and acini of the mammary glands of the male dog were stimulated by stilbestrol to tremendous hyperplasia with consequent mammary enlargement, shown in these and previous [1] experiments, some regression occurred in a short time, as illustrated by dogs S-C and S-A, and a normal condition was finally attained in dog 52.

At the height of the medication, all dogs except S-E showed a reduction in the level of hemoglobin varying from 12 to 21 per cent and of erythrocytes from 12 to 27 per cent. These reductions may have furnished a stimulus to the bone marrow for the production of erythrocytes. The normoblastic hyperplasia in the marrow of dogs S-C, S-A, and 52 could have resulted from such a stimulus. The fortuitous acute prostatitis in dog S-A was probably responsible for the discrepancy in its terminal marrow picture as compared with the other three dogs. The stimulus of the acute inflammatory process could have outweighed that for the regeneration of erythrocytes, so that the net result was an increased myeloid/erythroid ratio, rather than a decreased ratio as seen in dogs S-F, S-C, and 52. The hypoplastic marrow of dog S-E was undoubtedly due to a high effective level of stilbestrol caused by kidney damage interfering with the excretion of the drug. References to the eventual exhausting effects on the bone marrow of the dog of large doses of estrogens given over a short period of time have been given.[8]

The only definite comment on the data in Tables III and IV which may be made at this time is that dog S-E showed severe atrophic changes in the follicles of the thyroid gland and a great increase in acidophils of the anterior lobe of the pituitary gland.

SUMMARY

Following the cessation of orally administered stilbestrol, residual histologic changes were observed in the adrenal glands, the prostate gland, and the bone marrow of male dogs. The adrenal cortex showed a delayed start in the evolution of the fascicular cells from the glomerulosal cells and an earlier degeneration than is normal. The ducts and acini of the prostate gland, especially those leading to the dorsal parts of the lateral lobes, were involved by persistent atrophy and

squamous metaplasia. Normoblastic hyperplasia was found in the bone marrow, probably in response to a decrease in hemoglobin and erythrocytes at the height of the medication. On the other hand, the testes, penile sheath, and mammary glands apparently returned to normal. Suggestive but inconclusive changes were present in the thyroid and pituitary glands.

Spontaneous renal disease produced profound clinical and anatomic changes in one dog and caused definite clinical symptoms in another, probably by interfering with the excretion of stilbestrol in the urine to the point of raising the amount in the blood to a much higher effective level than possible in dogs with normal kidneys and comparable dosages.

Other organs failed to reveal residual effects attributable to stilbestrol.

REFERENCES

1. Mulligan, R. M. Feminization in male dogs. A syndrome associated with carcinoma of the testis and mimicked by the administration of estrogens. *Am. J. Path.*, 1944, 20, 865–875.
2. Mulligan, R. M. Connective tissue neoplasms produced in dogs by methylcholanthrene. *Proc. Soc. Exper. Biol. & Med.*, 1944, 57, 134–135.
3. Zondek, B., and Sulman, F. Inactivation of diethylstilbestrol in the organism. *Nature, London,* 1939, 144, 596–597.
4. Longwell, B. B., and McKee, F. S. The excretion of estrogens in the bile and urine after the administration of estrone. *J. Biol. Chem.*, 1942, 142, 757–764.
5. Glass, S. J., Edmonson, H. A., and Soll, S. N. Sex hormone changes associated with liver disease. *Endocrinology*, 1940, 27, 749–752.
6. Morrione, T. G. Effect of estrogens on the testis in hepatic insufficiency. *Arch. Path.*, 1944, 37, 39–48.
7. Smith, K. W., and Jensen, R. Personal communication.
8. Mulligan, R. M., Longwell, B. B., and Morrell, R. M. The tissue changes produced by estrone injected into female dogs with bile fistulas. *Am. J. Path.*, 1943, 19, 861–871.

DESCRIPTION OF PLATE

PLATE 49

FIG. 1. Cross section of an adrenal gland of dog S-E, showing the cortex to be widened and greatly depleted of lipids. Sudan IV stain. × 8.

FIG. 2. Cross section of an adrenal gland of dog S-F, showing a greatly narrowed and wrinkled cortex. Sudan IV stain. × 8.

FIG. 3. Cross section of an adrenal gland of dog S-C. The cortex is partly scalloped and the zona reticularis is widened. Sudan IV stain. × 8.

FIG. 4. Cross section of an adrenal gland of dog S-A, showing widening of the zona reticularis. Sudan IV stain. × 8.

FIG. 5. Cross section of an adrenal gland of a control dog. The width of the cortex is fairly uniform. The zones are discrete, and the lipid is evenly distributed. Sudan IV stain. × 8.

FIG. 6. Kidney of dog S-E, showing tangled masses of spirochetes and a few discrete organisms in a convoluted tubule in the upper half of the field. Dieterle stain. × 1100.
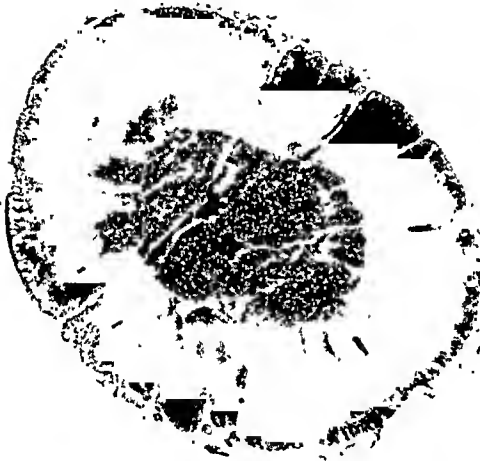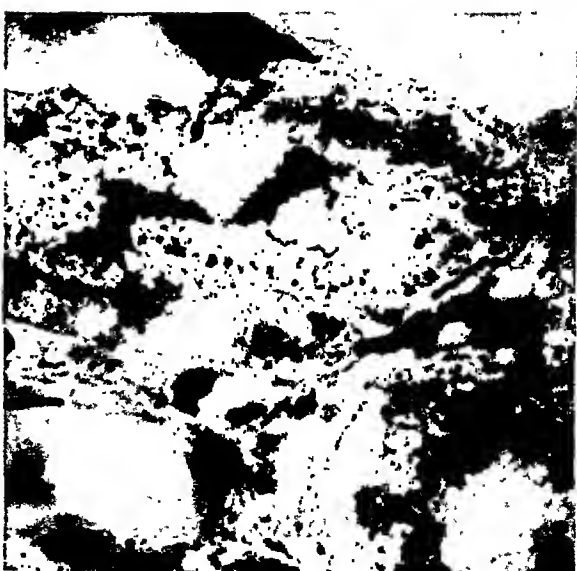
Mulligan and Becker   Male Dogs Following Cessation of Stilbestrol

STUDIES ON THE MOTOR CELLS OF THE SPINAL CORD

V. Poliomyelitic Lesions in the Spinal Motor Nuclei in Acute Cases [*]

H. Chandler Elliott, Ph.D.

(From the Department of Anatomy, Medical College of the State of South Carolina, Charleston, S.C.) [†]

In a preceding paper,[1] I described the defects left by poliomyelitis in the spinal motor nuclei of convalescent and chronic cases. Six specimens were discussed, five human and one macaque. It was found that the disease apparently invades the ventral gray column from a dorsomedial direction, for small lesions were always confined to dorsomedial groups of cells, whereas large lesions spared only ventrolateral groups. Also, when a lesion expanded from level to level it always did so in a ventrolateral direction. These generalizations were confirmed by every focus of infection—about twenty of them—in all six subjects.

Such findings are of obvious interest. But even the most uniform findings from six specimens, although they may strongly indicate a trend, are hardly adequate for final conclusions. Yet cords from chronic cases are hard to obtain; a continent-wide canvass, generously aided by hundreds of persons and institutions, secured only the five human specimens mentioned. It would, of course, be possible to prepare more monkeys, but, although the one examined gave interesting supplementary evidence, results from these animals cannot be applied to man with confidence. For example, Howe and Bodian [2] have shown that a nasal route of infection produces typical symptoms in the macaque; but there is no conclusive evidence that this portal is utilized in man. The portal could have an important bearing on the distribution of lesions.

From the same canvass, however, over thirty cords from acute human cases were collected. Difficulty was anticipated in determining areas of cellular destruction and survival. Other features of the inflammatory reaction, such as infiltration, would mask damage to the motor cells. Also, these cases, having terminated fatally, supposedly would tend to show more widespread destruction without discrimination between nuclei. But examination of the material proved these difficulties to be less formidable than expected, and also revealed interesting unforeseen data. Most of these cords from acute cases were

successfully studied by the methods used on the preceding series,[1] and the results are presented here.

LITERATURE

In the paper already cited,[1] the few existing references bearing on localization of lesions were enumerated and discussed. In brief, Schwalbe[3] and Horányi-Hechst[4] gave data on nuclear lesions, suggesting a dorsolateral origin for invasions of the ventral columns. Hurst[5] and Peers[6] made incidental comments supporting the same belief. Horányi-Hechst, in particular, presented a large group of cases (38), but his localizations were necessarily rather rough, since he had no reliable map of the motor nuclei. Charting of the motor nuclei in the cord by Romanes[7] and by me[8,9] opened the way for a more precise study of the lesions. Discussions of the general pathology of the disease, without reference to localization, form a long bibliography, but have no bearing on the present problem.

Since collecting these references, I have read with interest the work of Cooper and Sherrington[10] on ventral horn cells in the monkey. These authors claim that certain marginal cells in the ventral column, closely resembling the motor neurons, are not motor cells after all (at least in the lumbosacral region of the macaque); rather, they are related to the dorsal spinal nucleus (Clarke's column) and give rise to spinocerebellar fibers. This finding is of interest in the present connection, since it might account for some cases of surviving marginal cells in regions of the ventral column otherwise denuded.

MATERIALS AND METHODS

Twenty-five cases are here summarized. For the majority, histories were not available, but all patients were known to have died from the disease itself or from complications setting in immediately after the acute stage and before inflammation had subsided. This was generally confirmed by microscopic examination. Such case histories as were given indicate subjects of both sexes and a range of ages from infancy to middle life. All cords available were used except two in which post-mortem degeneration obscured the picture and three which were represented only by fragments in which lesions were not found. Thus the cases are not selected.

The lumbosacral and cervical regions alone were studied, since only in them are there groups of several cellular nuclei among which the disease could discriminate.

The methods employed were exactly the same as those described in earlier papers of this series;[1,8,9] i.e., mounting in complete serial sec-

tions, staining with toluidine blue, and preparing composite nuclear charts with a projector (see especially [8]). In the present study the only complication encountered was difficulty in determining the presence of normal cells surviving in areas of inflammation; the rule, then, was to disregard any cell of questionable normality. If strictly applied, this could favor no hypothesis, but a certain amount of personal judgment naturally was involved. Hence, two other operators, Dr. D. R. Noble and Miss Betty Bates, were engaged to rechart some of the doubtful areas. They, with no knowledge of my opinions, or other preconception, nevertheless produced results indistinguishable from mine.

OBSERVATIONS

As in my earlier papers, a general commentary will precede the individual protocols. The latter have little significance unless seen in relation to the whole picture.

The findings overwhelmingly confirm the thesis already presented [1] that lesions appear to begin dorsomedially and to spread ventrolaterally. Furthermore, extreme caudal groups also are frequently spared.

A few minor exceptions to the first rule were found. These require special comment and hence tend to assume a disproportionate prominence. It cannot be too strongly emphasized that, even when taken uncritically, they implicate only 16 per cent of the subjects. But when critically examined, many can be reasonably explained (see Discussion), and true exceptions involve only 2 or 3 per cent of lesions.

The degree of involvement varied greatly, which is surprising since all cases had ended fatally. Two or three cases, not mentioned below, showed no visible lesions in the material available. Beyond these a graded series can be traced through cases with small, localized foci of destruction, up to those with almost complete loss of motor cells. Thus, the findings are based on observation of all phases of invasion.

No study was made of the levels at which lesions were found. The material was not adequate for statistical treatment, and in most specimens the pathologic processes were too diffuse to permit one to assign them to any limited level. Similarly, no data were acquired on nucleus-muscle relationships, although this was an important secondary interest in preceding papers, for, even when records are available, patients in the acute stage of the disease do not generally display isolated muscle defects which could be correlated with the nuclear findings.

The following protocols are arranged in order numerically, as the specimens were received. (Missing numbers refer to cords previously discussed,[1] to those showing no lesions, and to other tissues not con-

cerned in this work.) Each description begins caudally and passes rostrally. Throughout, the term "cell" should be understood as referring only to motor cells of the ventral column. For nuclear numbers, see Text-Figure 1.

Case 42

Female, about 15 years of age. Fragments only were available. The largest portion, about L5-S1, showed an extensive lesion sparing only nucleus no. 6 and a few cells apparently from nos. 3 and 4. Other sacral and cervical portions showed no lesion. This finding is in exact accord with the general thesis.

Case 66

Sacral and mid-cervical regions were available. Sacral: Only no. 1 and the caudal part of no. 2 survived. The lesion invaded no. 2 progressively from the dorsomedial aspect as one progressed rostrally and obliterated it within about 1 mm. Cervical: Only a few groups of cells survived at the extreme lateral tip of the horn. In this specimen, cells could be seen in progressive stages of degeneration, from slight chromatolysis to replacement by clumps of phagocytes. Severity of these phenomena increased dorsomedially. These findings are in exact accord with the thesis. The observation on graded degree of degeneration is of corroborative value.

Case 69

Sacral and cervical regions were available. Sacral: At most levels the nuclei were completely destroyed. About caudal S1 no. 6 appeared, followed in order by nos. 4, 3, 5, and 7; about mid-S1 these groups disappeared again in reverse order. One small group was seen laterally and one ventrally; in the absence of other groups the levels could not be determined satisfactorily. These findings are in exact accord with the thesis.

Case 71

Sacral and mid-cervical regions were available. Sacral: Only ventral marginal groups of cells survived. On the right, no. 2 appeared caudally, but faded out from the dorsomedial side. Cervical: A few small groups survived, some laterally, some ventrally. These findings are in exact accord with the thesis.

Case 74

Lumbosacral enlargement was available. Caudally there was a typical cell pattern. About rostral S2 the medial groups disappeared; no. 6 (lateral) and nos. 3 and 4 (ventral) survived irregularly to rostral S1. In the lumbar segments only a few marginal cells survived. These findings are in exact accord with the thesis.

Case 75

Caudal L5 to rostral L2, mid-T1 to mid-C7, and mid-C6 to mid-C5 were available. Lumbosacral: No lesions were observed. Cervical: The first fragment showed erosion of medial nuclei on both sides, varying considerably from level to level, but probably involving nuclei nos. 12, 13, and 15 in different proportions. The second fragment showed a diffuse lesion, highly variable from level to level, but with surviving ventral cells even where erosion was most marked. These findings are in exact accord with the thesis.

Case 78

Caudal S1 to caudal S2 and most of the cervical enlargement were available. Lumbosacral: No lesions were visible. Cervical: The cell pattern was obscured by infiltration so that levels could not be determined. Nevertheless, through large
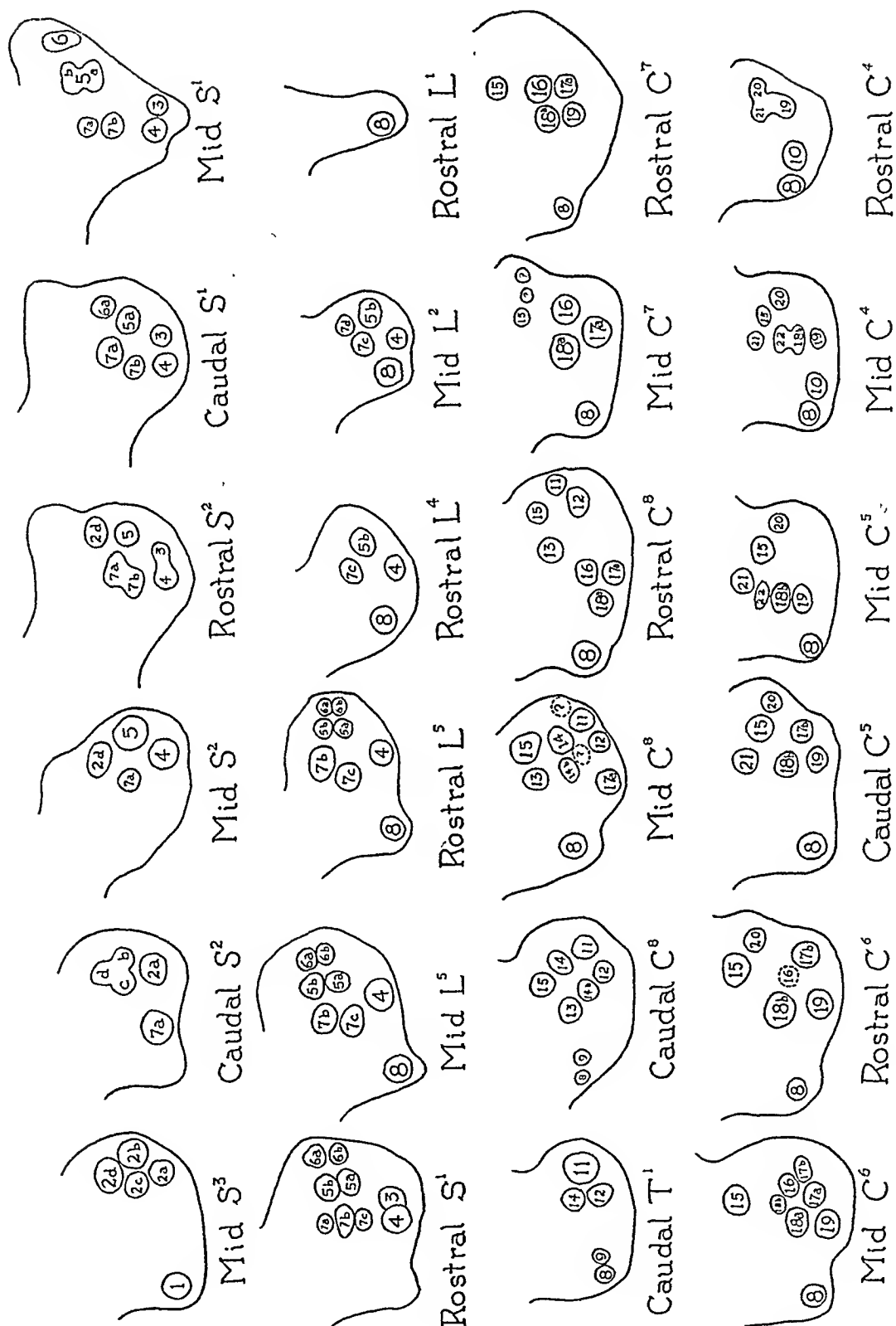
Text-Figure 1. Diagrams of the ventral gray column cut in transverse section at the levels indicated. (Reproduced by permission from *The American Journal of Anatomy*, 1943, **72**, 29–38.)

parts of the material a dorsomedial breakdown was evident, ventral or lateral groups alone surviving at some levels. Because of the diffuse inflammation it was impossible to exclude certain groups of cells as exceptions to the rule, but neither was it shown that they were exceptions. These findings are in accord with the thesis.

Case 80

Lumbosacral and cervical regions were available. Cell destruction was almost complete throughout. Only small groups of lateral, and a very few ventral, cells survived in either region. All stages of degeneration, from slight chromatolysis to complete destruction marked by groups of phagocytes, could be seen. The degree of destruction increased dorsomedially. At one point in the lumbar region (exact level not determinable) an almost complete nuclear pattern survived for a few sections. These findings are in exact accord with the thesis. The atypical level emphasizes the danger of drawing conclusions from single sections.

Case 81

Lumbosacral and cervical regions were available. Cell destruction was almost complete throughout. A few ventral and lateral cells survived. All stages of degeneration, from slight chromatolysis to complete destruction marked by groups of phagocytes, could be seen. Degree of destruction increased dorsomedially. One small group of cells, probably part of no. 21, survived dorsomedially. These findings agree, for the most part, with the thesis, but the survival of a dorsomedial group is exceptional.

Case 82

Lumbosacral enlargement was available. Nuclei nos. 1 and 2 (caudal) were intact. Nos. 3 and 4 (ventral) and 6 (lateral) appeared irregularly on both sides. The other nuclei were destroyed except for a short distance where the whole pattern appeared intact. These findings are in exact accord with the general thesis. The intact level provides another example of the hazard of judging from single sections.

Case 83

From a white female, 6½ years of age, the cervical enlargement was available. Medial groups were irregularly eroded throughout. At one level this erosion expanded ventrolaterally to include almost the whole lateral cell mass through perhaps 1 mm. of cord, and then receded dorsolaterally. These findings are in exact accord with the thesis.

Case 84

Lumbosacral and cervical enlargements were available. Lumbosacral: No trace of lesion was found. Cervical: An unusual type of lesion was present. There were scattered small foci of infiltration and of cellular destruction, which did not always coincide; i.e., many apparently normal cells were seen amid heavy inflammatory reaction, and cell destruction was evident where there was no infiltration. The great preponderance of destruction was medial, and of survival, lateral. One small group of cells, probably part of no. 15 (dorsomedial) survived in an otherwise denuded region. These findings, for the most part, are in accord with the thesis, but the survival of a dorsomedial group is exceptional.

Case 85

Lumbosacral and cervical enlargements were available. Cell destruction was almost complete throughout. All stages of degeneration, from slight chromatolysis to complete destruction marked by groups of phagocytes, could be seen. The degree of destruction increased dorsomedially. At a few levels more of the nuclear pattern appeared to survive, but it was much obscured by infiltration. These findings are in accord with the thesis, so far as could be determined.

Case 87

From a white female, 16 years old, lumbosacral and cervical regions were available. Lumbosacral: No cellular destruction was seen. Small foci of infiltration lay medial to the motor nuclei. Cervical: From T1 to caudal C5 there were slight medial erosions. More rostrally, the nuclei on both sides disappeared progressively, the process moving in a lateral direction, until in C4 only scattered lateral cells could be found. These findings are in exact accord with the thesis.

Case 88

Mid-S2 to caudal L5, caudal L3 to caudal L4, and most of the cervical enlargement were available. Lumbosacral: No lesions were visible. Cervical: From caudal T1 to about caudal C7 there was a very typical bilateral lesion, with survival of ventral and lateral groups. A small dorsomedial group, perhaps part of no. 15, also survived. More rostrally there was a less definite, diffuse, dorsomedial erosion. These findings are in accord with the thesis, excepting the small aberrant group.

Case 89

Caudal S1 to mid-L5 and an unidentified cervical fragment were available. Lumbosacral: No lesions were visible. Cervical: There appeared to be slight erosion dorsomedially on both sides. These findings are in accord with the thesis.

Case 90

Mid-S1 to mid-L5, L2, and three unidentified cervical fragments were available. Lumbosacral: No lesions were visible. Cervical: The nuclear pattern was very indefinite. A vague impression was received of erosions medially and dorsomedially. These findings are in accord with the thesis.

Case 91

Most of the lumbosacral cervical enlargements were available. Lumbosacral: Very typical lesions were found on both sides. Most of the nuclei were destroyed with survival of lateral and ventral groups at irregular levels. Cervical: Here the lesions also were typical. There was complete destruction generally, with survival of lateral groups at irregular levels. These findings are in exact accord with the thesis.

Case 93

Lumbosacral and cervical enlargements were available. Cell destruction was very slight. A few medially placed foci of infiltration appeared. In the cervical region there was apparent chromatolysis of cells in nuclei nos. 13, 15, and 21 (dorsomedial). These findings are in accord with the thesis, so far as they go.

Case 95

The lumbosacral enlargement was available. There was heavy infiltration with massive hemorrhage centrally in the lateral cell mass on both sides. The caudal nuclei were destroyed. Ventral and lateral cell groups survived, a large number of cells remaining apparently normal in spite of the close proximity of the massive lesion. A few medial cells, apparently from no. 7, also survived. These findings, for the most part, are in accord with the thesis, but the survival of medial cells and the destruction of the caudal groups are exceptional.

Case 97

Most of the lumbosacral enlargement and a small cervical fragment were available. Lumbosacral: The lesions were very typical, with no. 7 (medial) absent on one side for some distance. At another level no. 5 (dorsomedial) appeared to be eroded, with no lesion of no. 7 (more medial). Cervical: The material was too

incomplete to permit definite conclusions. These findings are generally in accord with the thesis. The lesion in no. 5 is unusual but hardly aberrant.

Case 98

A white male, 25 years of age, died from respiratory paralysis 2 weeks after onset. Lumbosacral and cervical enlargements were available. Lumbar: The picture was much obscured by infiltration. A dorsal group, probably no. 5, was missing at some levels. Other lesions were suspected, but not confirmed. Cervical: The medial nuclei, nos. 18 and 19, faded out bilaterally for some distance. These findings are only partly in accord with the thesis. Nos. 18 and 19 lie ventral as well as medial to the surviving groups, and so their involvement is to some extent exceptional, but the loss of no. 5, as in case 97, is hardly aberrant.

Case 99

Mid-S1 to caudal L5, and an unidentified cervical fragment were available. Lumbosacral: A lateral group alone survived on one side, a medial group alone was eroded on the other. Cervical: Scattered cells and groups of cells survived ventrally and laterally. Where these groups expanded they did so in a dorsomedial direction. These findings are in exact accord with the general thesis.

Case 100

Lumbosacral enlargement was available. The caudal groups were intact. About mid-S1, erosion began medially and involved nuclei nos. 7, 5, 4, and 3, in that order. No. 6 was spared throughout. These findings are in exact accord with the thesis.

Case 102

From a white female, 14 years old, lumbosacral and cervical enlargements were available. Cell destruction was almost complete throughout. In the lumbosacral region about 50 ventrolateral marginal cells survived on each side; these were scattered. In the cervical region, two lateral groups of about 100 cells each were seen. These findings are in exact accord with the thesis.

DISCUSSION

We have now to consider (1) how conclusive these findings are, (2) the bearing on them of Cooper and Sherrington's [10] marginal spino-cerebellar relay neurons, and (3) the possible significance of the dorso-medial position of the lesions.

(1) Thirty-one cases (including 6 in the preceding paper), if unanimous in their evidence, would be practically conclusive proof for the thesis. The question is whether the exceptions noted (in cases 81, 84, 88, 95, and 98) seriously compromise this proof. They implicate 16 per cent of the subjects. This is not a large proportion, as pathologic data go. Furthermore, in three of these (cases 81, 84, and 88) the exception comprises only one small dorsomedial group (possibly the same in all specimens); the overwhelming mass of findings from these cords supports the thesis. In case 95 the atypical massive hemorrhage throughout a great part of the ventral column might well have been expected to disrupt normal relationships. Case 98 was aberrant only in the cervical region, the lumbosacral region giving a picture in exact accord with the thesis. Thus, to reckon nonagreement at 16 per

cent is an exaggeration. It is not easy to set an accurate lower figure, but if we count lesions rather than subjects 2 or 3 per cent would more nearly indicate the extent of nonagreement.

In support of the findings can be cited also the 38 cases of Horányi-Hechst.[4] These were not studied and described in such detail as mine; in the absence of a dependable nuclear map this author could localize the lesions only in general terms. But the evident care and scholarship of his work inspire confidence in his findings. They agree entirely with mine. There are also scraps of evidence from the other authors cited, all of which support a dorsomedial center for the lesions.

Consistency so great as this is not only convincing, it is even surprising, especially in view of the many factors involved in a disease process. The force determining the dorsomedial origin of the lesions is evidently very potent and dominates other factors that might tend to modify or deflect it.

How to reconcile this great regularity in the sequence of involvement of nuclei, with the capricious pattern of muscular paralysis, was discussed in the preceding paper.[1] To recapitulate briefly: On the one hand, muscular involvement is by no means as irregular as appears at first sight; certain muscles are notoriously liable to paralysis, while others are rarely affected. On the other hand, even the regular picture of lesions of the spinal cord, as revealed here, allows for considerable variation; e.g., as to the level of the focus from which a lesion spreads, as to the degree of spread, and as to which dorsomedial nuclei are most severely involved.

(2) The claim that certain marginal cells belong to a spinocerebellar system in no way conflicts with the present thesis, but at most deprives it of some minor items of evidence. In some cases (74, 81, 102) it could account for neurons surviving in otherwise completely denuded regions. According to this belief, these cells, like the similar cells in the dorsal nucleus (Clarke's column), are not susceptible to the virus.

On the other hand, the present findings do offer some indirect evidence that marginal spinocerebellar neurons, as described in the macaque, exist also in man. The occasional survival of a rim of well preserved cells in an otherwise devastated region suggests that they differ in nature from the neighboring motor cells. In the majority of cases, however, surviving marginal cells cannot be explained as belonging to the spinocerebellar system. Groups found in the cervical region and those extending somewhat into the horn do not correspond to those described,[10] which are lumbosacral and strictly marginal only. Survival in other regions must be accounted for otherwise.

(3) Discussion of the significance of these findings is more fully

justified by their consistency in 31 cases than in 6. The immediate practical question is: Why do the lesions lie dorsomedially? A satisfactory answer to this would greatly clarify the natural history of the disease. In particular, it would provide direct evidence as to the paths followed by the virus in the central nervous system, and this in turn might indicate the portal of entry in man. More immediately, knowledge of the paths would offer a basis for rational prognosis and treatment.

Explanations of the phenomena described above, although necessarily speculative at present, may serve as a prospectus for further studies. Three theories have suggested themselves:

A. The appearance of the lesions, and of the surviving cells, strongly suggests that some groups are more vulnerable because they are nearer to some source of infection from which the virus radiates. This source might be any structure entering the ventral column dorsally or medially. Obvious candidates are the ventral arteries (which, in spite of their name, penetrate almost to the central canal before ramifying), primary or secondary fibers from the dorsal roots, the lateral corticospinal tract, the fasciculi proprii, and various minor tracts. In its simple form this theory is unsatisfactory. The arteries can be definitely excluded. The fact that an artery enters the column nearer to some nuclei than to others has no bearing on the matter. Virus, if carried by the blood, would diffuse almost exclusively through capillary walls and the relation of the capillaries to the cells is presumably much the same in all nuclei. Furthermore, personal observations did not show relationship between foci of infection and any feature of the arterial system. In any case, there is no proof that the virus of poliomyelitis is distributed to the nervous tissues by the blood.

As to the nerve tracts, the point at which the fibers entered the gray matter would likewise not be an important factor. Howe and Bodian [11] have shown that virus is transmitted along peripheral nerves at a rate of about 2.4 mm. an hour, and there is no reason to think that the rate would be much different in the axons of the central nervous system. The difference in position between medial and lateral nuclei is only 1 or 2 mm., implying a delay of less than 1 hour between the time when the virus reached the nearest and the farthest of them. This is not adequate to account for one being spared while the other is destroyed.

B. It is possible that cells in different nuclei have different susceptibilities. Howe and Bodian [12] showed that some nuclei of the nervous system are highly vulnerable to the virus, while others are almost immune. These authors have further demonstrated [13] that a slight

change in constitution, imperceptible to inspection, such as that persisting for some time after severance of the axon, may render a cell immune. And the cells of the dorsal nucleus, and the marginal cells discussed above, although closely resembling the motor neurons, are not commonly affected.

I am not inclined to favor this theory. Even the most ventral and lateral of the nuclei succumb often enough to indicate that their immunity, if it exists, must be very slight. Again, the theory does not explain why not only dorsomedial nuclei but sometimes even dorsomedial parts of individual nuclei tend to be attacked first, nor why a gradient of cell destruction can be found in many cases (66, 80, 81, 85). Certainly, immunity does not correspond with any observed characteristics of the cells; thus, those in nuclei nos. 3 and 4 are relatively small, those in no. 6 are large, but all tend to survive.

C. An interesting possibility lies in the fact that all motor nuclei have not the same connections. For example, those supplying extensor muscles must receive rich communications mediating extensor rigidity from the vestibular or reticular nuclei; flexor nuclei will receive secondary pain fibers from the dorsal horn, mediating the flexor reflex; and so on. Among these and many similar facts may lie the clue to the differential infection of nuclei. If the virus enters by a given portal, it will follow certain paths preferentially, and so reach certain nuclei first. This theory has the added advantage of permitting us to explain the frequent survival of the caudal nuclei and of the small dorsomedial group in the cervical region (cases 81, 84, 88). In that case it would appear that the dorsomedial beginning of the lesions is purely fortuitous—that the virus travels along tracts terminating on nuclei that simply happen to lie dorsomedially.

As with the preceding theory, one may object that not only ventrolateral nuclei, but also ventrolateral cells in those nuclei tend to be spared. It seems unlikely that some cells in a compact group have connections differing from others, but in the present case a plausible explanation suggests itself. We can assume that the virus reaches the dorsomedial nuclei directly, via some fiber tract; and, in fact, these innermost centers, when affected at all, seem to be completely destroyed. The virus could then spread to adjoining nuclei, but since there are no known internuclear fibers, this spread would probably be by diffusion through the tissue fluid and such a mechanism would be slow enough to account for some cells being destroyed before others.

Besides these three problems, one observation demands attention since it may prove to be very significant. In many cases the disease process appears to start from a very small number of foci, as few as

one or two. Where destruction has become general it is, of course, impossible to estimate the number of foci. But I would hazard an opinion that it is never very great. Evidently a single fiber, or small group, may suffice as a path for the virus; and evidently the resulting lesion, although it may spread greatly, does not metastasize.

The next step in this investigation is obviously to determine which spinal nuclei correspond to particular muscles. When this is done we can tell whether the most frequently affected centers do correspond to any neuromuscular system with definite reflex connections. This in turn might suggest portals of entry. It would also open the way for correlation of clinical and post-mortem findings, and assessment of therapy on experimental subjects. Experiments in this direction are in progress.

SUMMARY

This work is supplemental to a preceding paper. It offers a larger series of cases, and a more detailed discussion of findings.

The motor nuclei of the limbs were studied in cords from 25 human subjects who died from poliomyelitis. In agreement with the preceding paper, almost all lesions involved dorsal and medial nuclei, and extended ventrolaterally only secondarily. Exceptions were found, but these were rare and trivial.

It was further found that caudal nuclei and one small dorsomedial group in the cervical region tend to survive.

The probable presence and survival of marginal spinocerebellar cells in the ventral horns does not invalidate the findings.

It is suggested that the differential infection of nuclei is due not to their proximity to a source of infection, nor to their own intrinsic susceptibility, but to passage of virus along tracts ending in certain nuclear groups, e.g., those controlling muscles involved in decerebrate rigidity, in the flexor reflex, etc.

As few as one or two foci of invasion may be found in a fatal case. Thus the virus may find sufficient passage in a single fiber.

The importance of clarifying neuromuscular relationships, in order that the findings may be applied clinically, is emphasized.

R. Smith, Dalhousie University, Halifax; and Dr. L. W. Smith, Temple University, Philadelphia.

REFERENCES

1. Elliott, H. C. Studies on the motor cells of the spinal cord. III. Position and extent of lesions in the nuclear pattern of convalescent and chronic poliomyelitis patients. *Am. J. Path.*, 1945, 21, 87–97.
2. Howe, H. A., and Bodian, D. Neural mechanisms in poliomyelitis. The Commonwealth Fund, New York, 1942, p. 97 *et seq.*
3. Schwalbe, E. Untersuchung eines Falles von Poliomyelitis acuta infantum im Stadium der Reparation. *Beitr. z. path. Anat. u. z. allg. Path.*, 1902, 32, 485–525.
4. Horányi-Hechst, B. Zur Histopathologie der menschlichen Poliomyelitis acuta anterior. *Deutsche Ztschr. f. Nervenh.*, 1935, 137, 1–54.
5. Hurst, E. W. A further contribution to the pathogenesis of experimental poliomyelitis: inoculation into the sciatic nerve. *J. Path. & Bact.*, 1930, 33, 1133–1143.
6. Peers, J. H. The pathology of convalescent poliomyelitis in man. *Am. J. Path.*, 1943, 19, 673–696.
7. Romanes, G. J. Cell columns in the spinal cord of a human foetus of fourteen weeks. *J. Anat.*, 1941, 75, 145–152.
8. Elliott, H. C. Studies on the motor cells of the spinal cord. I. Distribution in the normal human cord. *Am. J. Anat.*, 1942, 70, 95–117.
9. Elliott, H. C. Studies on the motor cells of the spinal cord. II. Distribution in the normal human fetal cord. *Am. J. Anat.*, 1943, 72, 29–38.
10. Cooper, S., and Sherrington, C. S. Gower's tract and spinal border cells. *Brain*, 1940, 63, 123–134.
11. Howe, H. A., and Bodian, D. *Loc. cit.*,[2] p. 22.
12. Howe, H. A., and Bodian, D. *Loc. cit.*,[2] p. 75.
13. Howe, H. A., and Bodian, D. *Loc. cit.*,[2] p. 175.

ALEUKEMIC MYELOSIS

Chronic Nonleukemic Myelosis, Agnogenic Myeloid Metaplasia, Osteosclerosis, Leuko-Erythroblastic Anemia, and Synonymous Designations *

Elwyn L. Heller, M.D., Marjorie G. Lewisohn, M.D., and William E. Palin, M.D.

(*From the Department of Pathology, University of Pittsburgh, and the Presbyterian Hospital, Pittsburgh, Pa.*)

The existence of a disease closely related to, or a variant of, myelogenous leukemia has been known for many years. It presents features sufficiently distinct in certain respects to have resulted in its segregation from leukemia by numerous investigators. Thus, its exact relationship to leukemia has not been fully clarified. In this paper we present the evidence forming the basis for our interpretation that the disease is fundamentally leukemic, an interpretation generally implicit in the designation, aleukemic myelosis. The uncertainty in the classification of this disease has resulted in a diverse and confusing nomenclature, as illustrated in Table I.

The disease is characterized clinically by a slowly progressive splenomegaly occurring in persons of either sex, usually within or beyond the fifth decade of life. There may be little impairment in the general health for many years, following which generalized weakness, anorexia, weight loss, ill defined aches and pains, and discomfort, occasioned by the greatly enlarged spleen, frequently occur. The liver may enlarge. As a rule there is no significant involvement of the lymph nodes. The blood picture varies within relatively wide limits. There is usually an anemia of mild to moderate severity often associated with the presence of immature red blood cells. The white blood count may be elevated, at times to a degree suggestive of leukemia. Smears of peripheral blood may reveal immature myeloid cells. However, the degree of leukocytosis and the immaturity of the white blood cells, as a rule, are not typically leukemic and some cases have no leukemoid features.

Within the past 2 years we have had the opportunity of observing three cases of the disease. In one, only the spleen was available for

* Received for publication, June 17, 1946.

pathologic study. Autopsy examination was performed in the other two cases. Repeated blood studies were performed in each case, although in case 3 observation of the blood by one of us was limited to a single examination.

<div align="center">

TABLE I

Terminology Referable to:

</div>

A. Leukemia

Aleukemic myelosis [1-7]
Aleukemic leukemia [8*, 9†]
Pseudoleukemia [10-12]
Chronic nonleukemic myelosis [13-15]
Osteosclerotic leukemia [16]
Atypical myelosis [17]
Megakaryocytic myelosis [18]

B. Anemia

Leukanemia [19-21]
Myeloid splenic anemia [22]
Leuko-erythroblastic anemia,[23] leuko-erythroblastosis [24]
Osteosclerotic anemia [25]

C. Spleen and/or Liver

Splenomegaly with anemia and myelemia [26]
Myelophthisic splenomegaly [27]
Aleukemic hepatosplenic myelosis [28]
Agnogenic myeloid metaplasia of the spleen [29,30]
Myeloid megakaryocytic hepatosplenomegaly [31]
Splenomegaly with sclerosis of the bone marrow [32]
Splenomegaly with myeloid transformation [33]
Splenomegaly with anemia [34]
Myeloid splenomegaly without myelocythemia [35]

D. Bone Marrow

Myelofibrosis [36,37]
Myelosclerosis [9,38]
Osteosclerosis [5,6,15,18,29]

    * Cases 1, 2, and 8.
    † Cases 8, 9, and 10.

<div align="center">

CASE I

</div>

Mrs. B. J., a white woman, 58 years old, was admitted to the Butler County Memorial Hospital, service of Dr. Carl Danielson, on January 26, 1944, complaining of loss of weight, anorexia, and vague abdominal pain of several months' duration. The onset of her illness had been gradual and the symptoms had become progressively more severe. The remainder of the history was not noteworthy. Physical examination revealed a poorly nourished, frail woman in no apparent discomfort. The physical findings were normal except for a moderately firm, smooth spleen which extended to the level of the umbilicus. The red blood cells were 3,260,000 per cmm.; hemoglobin, 58 per cent; white blood cell count, 20,000 per cmm.; neutrophils (unclassified), 65 per cent; lymphocytes, 33 per cent; monocytes, 2 per cent. After a blood transfusion the erythrocytes rose to 3,640,000 per cmm., and the hemoglobin to 63 per cent. A second white blood cell count was 12,700 per cmm. On February 8, 1944, splenectomy was performed. The postoperative course, during which time she received 2,000 cc. of blood by transfusion, was uneventful. She was discharged on March 3, 1944.

<div align="center">

Post-Mortem Findings

</div>

The spleen was uniformly enlarged. It weighed 1,000 gm. and measured 24 by 13 by 9 cm. The organ had a firm consistency and was reddish brown, mottled with small darker hemorrhagic areas. On section, the parenchyma bulged slightly and was dark red, firm, and rela-

tively bloodless. There were numerous small, slightly elevated, irregular, hemorrhagic, nodular areas generally less than 1 cm. in diameter (Fig. 1). The lymphoid follicles were indistinct. Beneath the capsule a sharply circumscribed nodule, 1.5 cm. in diameter, was noted. Its cut surface was pale yellow, solid, and irregularly hemorrhagic.

Microscopic Examination

Sections of the spleen revealed an extensive pleomorphic cellular infiltration which had caused wide separation and atrophy of the lymphoid follicles. Throughout the pulp were innumerable myeloid cells at various stages of maturity from myeloblasts to segmented polymorphonuclear leukocytes. The sinusoidal spaces contained similar cells in small numbers. Scattered mitotic figures were noted in the immature cells. The lining reticulo-endothelial cells were enlarged and in areas appeared to be in process of separating from the underlying reticulum. Large multinucleated giant cells of megakaryocytic type were noted in the sinusoidal spaces, frequently lying on the reticulum of the wall. They possessed an abundant acidophilic nongranular cytoplasm and dark nuclei, multiple or coarsely lobate. Smaller, poorly developed forms resembled stages of transition from the hyperplastic reticulo-endothelial cells. In some areas nucleated red blood cells were relatively numerous. The nodules noted grossly were not encapsulated and consisted chiefly of tightly packed myeloid cells, including numerous giant cells (Fig. 2).

Postoperative blood studies are collected in Table II. A notable feature of the blood smears was the presence of giant platelets, frequently larger than the erythrocytes (Fig. 3). They were often hypochromatic and at times exhibited central vacuolization.

Additional laboratory studies revealed normal values for bleeding and clotting time, clot retraction, blood sugar, nonprotein nitrogen, serum phosphorus, and serum calcium. Sedimentation rate was 32 mm. in 1 hour; hematocrit, 32 per cent; hemolysis began at 0.48 and was complete at 0.34. The icterus index was 10 units; the reticulocyte count was 3.7 per cent. Serologic tests for syphilis were negative. Urine specimens were normal.

Roentgenograms of both femora revealed a normal texture.

At the present time, about 2 years following splenectomy, the patient is in poor health, having derived no apparent benefit from the operation. A recent radiologic examination revealed irregular areas of osteosclerosis involving the ribs, clavicles, and heads of the humeri. There has been no material change in her complaints, except for the occurrence of moderately severe epistaxis occurring at monthly intervals.

CASE 2

Mrs. A. B., a white housewife, 59 years old, was admitted to the Allegheny General Hospital, service of Dr. C. W. W. Elkin, on September 11, 1938, complaining of pain in the left upper abdominal quadrant and a palpable mass in that area which had been present, to the patient's knowledge, for 12 years. She also complained

TABLE II

Studies of the Blood in Case I

Date	2-14-44	2-19-44	2-20-44	2-21-44	2-22-44	2-23-44	2-26-44	4-6-44	5-11-44	3-28-45	1-30-46
				Postoperative							
Red blood cells (millions)	3.250	2.350	2.900	3.000	3.050	3.600	3.390	3.500	3.050	3.150	2.180
White blood cells	12,700	32,700	34,600	28,200	25,600	26,800	25,400	40,000	51,250	58,800	50,400
Hemoglobin in %; 14.5 gm.=100%	55	46	52	54	56	69	62	58	52	58	47
Platelets	264,750	164,500	201,800	270,000	279,750	306,500					
Differential counts											
Segmented polymorphonuclear leukocytes	16	8	7	7	7	4	7	7	4	5	2
Rod-nuclears	42	29	52	52	52	40	58	58	33	23	39
Metamyelocytes	6	17	16	16	16	30	21	21	13	18	18
Myelocytes	12	11	7	7	7	15	4	4	9	20	9
Premyelocytes	5	1	1	1	1	0	0	0	0	2	9
Myeloblasts	0	0	0	0	0	0	0	0	6	10	8
Lymphocytes	18	34	15	15	15	10	10	10	29	20	8
Eosinophils	1	0	1	1	1	0	0	0	2	2	5
Monocytes	0	0	1	1	1	1	1	0	4	0	2
Normoblasts	1	6	76	2	49	14	3	3	19	10	41
Megaloblasts			0	0	0	0	0	0	0	0	0

of mild intermittent precordial pain and slight exertional dyspnea of long duration, and generalized "neuritic" pains. The remainder of the history was irrelevant.

Physical examination revealed a well nourished, well developed woman, not appearing ill. The temperature and pulse were normal. Blood pressure was 140/70 mm. Hg. There was a systolic murmur over the precordium. The spleen was considerably enlarged, extending slightly beyond the midline and below the level of the umbilicus. It had a firm consistency and a somewhat rounded edge. The lower border of the liver extended from 3 to 4 fingersbreadth below the costal border. It was smooth and firm. There was no palpable enlargement of the lymph nodes.

The patient would not submit to biopsy of the sternal marrow and was discharged after 9 days with the diagnosis undetermined.

Roentgenograms of the chest and abdomen were noncontributory. The electrocardiogram was normal except for left axis deviation.

Laboratory Findings. The red blood cells were 4,500,000 per cmm.; hemoglobin, 70 per cent (10.5 gm.); white blood cell counts were 8,500 and 8,800 per cmm.; differential count: segmented polymorphonuclear leukocytes, 51 per cent; nonsegmented neutrophilic leukocytes, 19 per cent, with occasional metamyelocytes representing the most immature cell type; eosinophils, 1 per cent; basophils, 4 per cent; lymphocytes, 24 per cent; monocytes, 1 per cent. The erythrocytes showed moderate anisocytosis, poikilocytosis, and occasional stippling; normoblasts were noted infrequently. The blood sugar and nonprotein nitrogen were normal. Serologic tests for syphilis were negative. The urine contained a faint trace of albumin and a few white blood cells.

Four and one-half years later, on March 4, 1943, the patient was admitted to the Presbyterian Hospital, service of Dr. C. W. Morton, with a severe infection of the upper respiratory tract. Physical findings were unchanged, except for a somewhat larger spleen.

Laboratory Findings. The red blood cells were 3,310,000 and 3,790,000 per cmm.; hemoglobin, 55 and 60 per cent; white blood cell count, 8,400 per cmm.; polymorphonuclear neutrophils, 44 per cent; lymphocytes, 48 per cent; monocytes, 8 per cent. She was discharged on March 9, 1943.

On February 12, 1945, 2 years later, she was readmitted to the Presbyterian Hospital complaining of weakness, weight loss, anorexia, nausea, vomiting, and vague abdominal distress. She appeared chronically ill. The spleen was massive and the liver was moderately enlarged. The physical findings were otherwise unaltered.

Laboratory Findings. The red blood cells were 2,480,000 per cmm.; hemoglobin, 40 per cent; several white blood cell counts varied between 5,400 and 16,300 per cmm. Average values for several differential blood counts were as follows: segmented neutrophilic leukocytes, 20.4 per cent; rod-nuclears, 30.5 per cent; metamyelocytes, 16.8 per cent; myelocytes, 8.6 per cent; eosinophils, 3.5 per cent; lymphocytes, 18.4 per cent; monocytes, 1.8 per cent; normoblasts, from 2 to 52 per 100 white blood cells; megaloblasts, 10 per 100 white blood cells in one smear.

Following a progressively downhill course with periods of intermittent fever and extreme weakness the patient died on March 14, 1945, 19 years after the splenic enlargement was first noted.

Post-Mortem Findings

Autopsy was performed 3 hours after death. External examination revealed nothing noteworthy. The lungs and heart were normal.

The spleen was enormously enlarged, extending beyond the midline and below the level of the umbilicus. It weighed 2,190 gm. and meas-

ured 30 by 18 by 8 cm. The capsule was irregularly thickened, but smooth. The organ was a mottled bluish purple and moderately firm. On section a dark red, fleshy parenchyma studded with small, poorly defined nodules was noted. The nodules were distinguished from the adjacent pulp by a firmer texture and slight elevation. A few were pale. There was no apparent encapsulation of the nodules and their borders merged imperceptibly into the adjacent pulp tissue. The lymphoid follicles were not grossly evident. Small areas of scarring were noted, but no infarcts.

The liver was enlarged and weighed 2,425 gm. It was firm and of a uniform light tan color. The lower border of the right lobe was somewhat rounded. On section the parenchyma was pale and free of localized lesions. There was no apparent disturbance in the portal architecture. The lymph nodes of the thorax or abdomen were not altered in size or appearance. The bone marrow of vertebrae, ribs, sternum, ilium, and neck of femur appeared cellular and somewhat paler than is normal.

No other significant gross abnormalities were evident.

Microscopic Examination

Spleen. An abundant myeloid tissue replaced much of the splenic pulp. The fibrous septa and lymphoid follicles were widely separated, the latter being small and compressed, but distinct. The intensity of the myeloid reaction varied. It occurred in a diffuse manner, but in some areas there were circumscribed nodular masses of closely packed myeloid cells which were not encapsulated. Numerous large, irregular giant cells of megakaryocytic type were conspicuous, generally located within the sinuses (Fig. 4). The cytoplasm of these cells was abundant, homogeneous, hyalin-like, nongranular, and intensely acidophilic. The nuclei were large, often huge, and generally oval or coarsely lobular, lightly basophilic and vesicular. Pyknotic forms were encountered at times. The majority of these cells were multinucleated, often containing a dozen or more nuclei. Present within the sinusoids, in large numbers, were irregular clusters of immature myeloid cells, myeloblasts predominating. The latter had a lightly basophilic, slightly granular cytoplasm. The nuclei were large, round, and vesicular with a prominent nuclear membrane enclosing a stippled chromatin structure. Prominent nucleoli and mitotic figures were frequent. More mature forms, exhibiting cytoplasmic granulations, both neutrophilic and eosinophilic, were in evidence. Polymorphonuclear leukocytes, lymphocytes, and scattered erythrocytes were present, in smaller numbers, in the sinusoids. In the interstitial tissue there were many erythrocytes, lympho-

cytes, and numerous relatively mature myeloid cells. Compressed masses of lymphoid tissue could be identified, representing remnants of the lymphoid follicles. Normal appearing reticulo-endothelial cells were occasionally encountered along the sinusoids. Many resembled developmental stages of transition into myeloblasts or megakaryocytes (Figs. 5 and 6). Their position upon the wall of the sinus was suggestive of their local origin from reticulo-endothelial cells. No erythropoietic activity was in evidence. Smears of fresh pulp made at the autopsy and stained with peroxydase and Wright's stain revealed no normoblasts or other erythropoietic cells. In addition to lymphoid cells and erythrocytes there were numerous myeloid cells, the majority containing scattered oxydase-positive granules.

Lymph Node. A peribronchial lymph node revealed extensive anthracotic pigmentation and fibrosis. Large, irregular, multinucleated giant cells resembling megakaryocytes were infrequent along the sinusoidal walls. There was no additional evidence of hematopoiesis.

Bone Marrow. Sections from the bodies of the lumbar vertebrae, sternum, ribs, ilium, and femur were examined. In all areas there was an intense pleomorphic myeloid hyperplasia which had replaced the marrow fat (Fig. 7). There were small, widely scattered areas of fibrosis which did not significantly alter the appearance of intense hyperplasia. Prominent throughout all areas were megakaryocytes, increased in number above normal. Generally they were located in irregular sinusoidal spaces. Immature myeloid tissue, in cellular nests, frequently distended the vascular spaces. The interstitial tissue was composed of closely packed myeloid cells in various stages of development, for the most part moderately well differentiated. In some areas eosinophilic myelocytes and metamyelocytes were unusually numerous. Mitotic figures were frequently noted in the immature cells. There was an obvious reduction, and in most areas complete suppression, of erythropoietic activity. Small clusters of normoblasts and megaloblasts were encountered occasionally.

Liver. Sections of the liver revealed a moderately advanced passive congestion. The portal areas showed no infiltration with myeloid cells. The lobular structure was well maintained. A moderately heavy, diffuse, myeloid involvement of the sinusoidal spaces was noted (Fig. 8). Elongated giant cells, usually multinucleated, were scattered in small numbers within the sinuses, generally lying near the sinus wall in endothelial fashion (Fig. 9). Myeloid cells in various stages of development were prominent in these areas.

Sections of thyroid, lungs, heart, gastrointestinal tract, kidneys, adrenals, pancreas, uterus, and tubes showed no myeloid involvement.

CASE 3

A. W., a white male, 73 years old, was admitted to the Western Pennsylvania Hospital, service of Dr. Frank A. Evans, on October 26, 1939, complaining of weakness, anorexia, weight loss, and mild dyspnea. The symptoms had been most pronounced for a month prior to admission, but their onset had been insidious and rather indefinite. In the preceding 3 years there had been a weight loss of 100 lbs.

Physical examination revealed a well developed, fairly well nourished patient in no acute distress. The skin and mucous membranes were pale. There was slight icteric discoloration of the skin and sclerae. There was no atrophy of the glossal papillae. No enlargement of the superficial lymph nodes was noted. The lungs were clear. There was a harsh systolic murmur noted at the apex and base of the heart. There was no apparent cardiac enlargement. Blood pressure was 130/85 mm. Hg. There were no abnormal findings referable to the abdomen. The spleen was not palpated. There was diminished vibratory perception in the extremities. No additional neurologic abnormalities were noted, other than partial deafness. Preliminary blood studies were highly suggestive of pernicious anemia, and intense parenteral therapy with concentrated liver extract was instituted. The anemia responded well to liver therapy supported by two transfusions of whole blood. The patient was discharged, considerably improved, on November 18, 1939.

Laboratory Findings. Examination of the urine and feces revealed nothing noteworthy. On admission the red blood cell count was 950,000 per cmm.; hemoglobin, 15 per cent; white blood cell count, 1,600 per cmm. The erythrocytes showed considerable anisocytosis and poikilocytosis. Nucleated red blood cells were noted. Differential examination of the leukocytes revealed: neutrophils, 46 per cent; small lymphocytes, 46 per cent; large lymphocytes, 5 per cent; monocytes, 3 per cent. The icteric index was 20. There was total absence of free hydrochloric acid in the gastric juice on two occasions. There was a progressive rise in the red blood cell count to 3,500,000 per cmm. at the time of discharge. The hemoglobin had risen to 71 per cent. The reticulocytes rose rapidly from an initial 0.2 per cent to a maximum of 12 per cent 13 days after the onset of liver therapy. There was a persistent leukopenia, the white blood cell count ranging between 1,600 and 4,600 per cmm., usually between 2,000 and 3,000 per cmm. There was no significant change in the differential percentages, and immature granulocytic forms were not noted. Normoblasts were recorded repeatedly.

Six years later, on August 30, 1945, the patient was admitted to the Presbyterian Hospital, service of Dr. W. A. Bradshaw. He complained of weakness and loss of weight, present intermittently since 1939. The weakness had diminished during periods of liver therapy until 3 months prior to admission.

Laboratory Findings. The red blood cell count was 1,780,000 per cmm.; hemoglobin, 37 per cent; white blood cell count, 10,800 per cmm. Differential count of 200 cells revealed: segmented neutrophils, 69.5 per cent; rod-nuclears, 14.5 per cent; metamyelocytes, 3.5 per cent; myelocytes, 3 per cent; lymphocytes, 7.5 per cent; monocytes, 1.5 per cent; basophils, 0.5 per cent; 16 normoblasts and 3 megaloblasts were noted. Gastric analysis revealed no free hydrochloric acid. Other examinations yielded nothing noteworthy.

Physical findings were essentially those of his former admission, and his general condition was poor. He was afebrile until the third day when he suffered a shaking chill with a rise of temperature to 105° F. He continued febrile and died 5 days after admission.

Post-Mortem Findings

Autopsy was performed 1½ hours after death. There were no significant external abnormalities. The superficial lymph nodes were not enlarged. The lungs showed congestion and irregular areas of broncho-

pneumonic consolidation in both lower lobes. There was moderate arteriosclerotic thickening of the leaflets of the mitral and aortic valves. There were 1,500 cc. of clear ascitic fluid in the peritoneal cavity. The surface of the liver was finely granular and mottled brown and yellow. It weighed 2,280 gm. It cut with a fibrous resistance. Its cut surface was delicately nodular and the lobular structure was not clearly evident. There was a delicate fibrosis throughout, the pale fibrous stroma contrasting with the brown parenchyma.

The spleen was considerably enlarged but did not extend below the lower costal border. It weighed 1,110 gm. and measured 20 by 16 by 6 cm. Its upper surface was densely adherent to the peritoneal surface of the diaphragm. The cut surface was uniform in appearance throughout; it was reddish pink, fleshy, and moderately firm. The lymphoid follicles were small and indistinct. Little blood escaped from the cut surface.

The paravertebral lymph nodes were moderately enlarged, measuring up to 4 cm. in diameter. On section the parenchyma was pale, glistening, translucent, and fleshy. The bone marrow of the vertebral bodies and sternum was red and grossly normal in appearance.

There were no significant alterations noted in the other abdominal viscera.

Microscopic Examination

Spleen. Throughout the splenic pulp there were large numbers of myeloid cells in various stages of development. Numerous myeloblasts were encountered in the sinusoids as well as throughout the interstitial tissue. A few mitotic figures were noted in them. Giant cells of megakaryocytic type, frequently multinucleated, were occasionally noted. They were generally located along the sinusoidal walls in endothelial fashion. The reticulo-endothelial cells were prominent and stages of transition into giant cells were encountered. There was no significant increase in the fibrous stroma. The septa and lymphoid follicles were widely separated. The follicles were frequently atrophic and irregularly infiltrated with myeloid cells (Fig. 10). Clusters of cells with small, compact, hyperchromatic nuclei and oxyphilic cytoplasm were noted infrequently; they were regarded as normoblasts.

Lymph Nodes. Sections of paravertebral lymph nodes revealed irregular myeloid transformation of the parenchyma, of slight extent in some nodes, of considerable extent in others. Myeloblasts, myelocytes, neutrophilic leukocytes, and intermediary forms resulted in a notable pleomorphism, accentuated by numerous giant cells within the sinusoidal spaces (Fig. 11). Morphologically, the latter cells were identical to those of spleen and liver but were much more numerous

than in those organs. The myelosis had replaced much of the lymphoid pulp, had distorted the cords and sinusoids, and compressed and infiltrated some of the lymphoid follicles. In the nodes exhibiting the most intense myelosis, however, subcapsular follicles of normal size and appearance were evident.

Bone Marrow. The marrow of the vertebrae and sternum was uniform in appearance. There was considerable reduction of the marrow fat resulting from intense myeloid proliferation (Fig. 12). Various types of myeloid cells were represented. Mitotic figures were occasionally noted in the more immature cells. Prominent in the reaction were numerous megakaryocytes, cytologically normal. Only small scattered foci of erythropoiesis were identified. There was no significant fibrosis of the marrow and the bony lamellae were normal in size and structure.

Liver. There was slight to moderate disturbance in the portal structure of the liver, and small rounded nodules of hepatic parenchyma, without a central vein and surrounded by thickened portal stroma, were frequently noted. The appearance was that of portal cirrhosis of only moderate extent. Irregularly distributed throughout the sinusoidal spaces were myeloid cells in various stages of development. There were many giant cells, frequently having large lobate or multiple nuclei, lying along the walls of the sinusoids. The Kupffer cells were prominent and double-nucleated forms were noted. Frequently the portal stroma was infiltrated with young myeloid cells (Fig. 13). No giant cells were noted in these areas. The hepatic cord cells generally appeared normal, but in some areas contained considerable fat.

Sections of thyroid, heart, lungs, gastrointestinal tract, kidneys, adrenals, pancreas, bladder, and prostate showed no myeloid reaction. No pathologic features suggestive of pernicious anemia were noted.

Morphologic Features

Inasmuch as cases such as we have reported have been interpreted by numerous investigators as representing a disease other than leukemia, it is necessary (1) to present the evidence for our interpretation as leukemia and (2) to invalidate the criteria which have served to differentiate the disease from leukemia. Although we shall refer to the disease under discussion as aleukemic myelosis, it must be emphasized that comments referable to this disease are generally applicable to cases described in the literature under the terminology of Table I.

In the final analysis, the pathologic features offer the strongest basis upon which to form conclusions regarding the nature of a disease process. The clinical and hematologic findings are a reflection of the basic tissue reaction.

The gross features of typical myelogenous leukemia are the result of myeloid proliferation, characteristically of bone marrow, liver, spleen, and, to a variable extent, in the lymph nodes. In aleukemic myelosis grossly similar changes are encountered with the qualification that the bone marrow of some cases may be hypoplastic or sclerotic.

Spleen

The spleen was considerably enlarged in all three cases. In two, nodules were visible over the cut surface. The lymphoid follicles were not distinct. Fibrosis was not a prominent feature. No infarcts were noted.

Jackson, Parker, and Lemon[29] considered the absence of splenic infarcts, so common in myelogenous leukemia, as a feature differentiating "agnogenic myeloid metaplasia" from the former disease. Such a criterion could hardly be considered as fundamental inasmuch as infarcts are variable lesions subject to conditions affecting the vascular supply. In a study of the spleen in 27 cases of chronic myelogenous leukemia, Krumbhaar and Stengel[40] recorded infarcts in only 9. Conversely, Churg and Wachstein[15] described infarcts in the spleen of a case of "chronic nonleukemic myelosis." In a case of "leuko-erythroblastic anemia with myelosclerosis," Vaughan and Harrison[38] noted a large infarct.

Microscopically, the pulp was largely converted into myeloid tissue in which all intermediary stages of development of the myeloid cells from myeloblasts to segmented polymorphonuclear leukocytes were recognizable. The diffuse myelosis and mitotic figures were impressively reminiscent of a genuine leukemic reaction. The myelosis had resulted in wide separation of the lymphoid follicles which, as a rule, were atrophic.

Generally, the lymphoid follicles of the spleen in myelogenous leukemia are destroyed by the exuberant myeloid proliferation. Their retention in aleukemic myelosis has served to differentiate it from leukemia. Obviously, the disappearance of the follicles is an atrophic and replacement phenomenon and entirely subject to the extent of the myelosis. That there is any fundamental distinction between incomplete and total myeloid conversion of the splenic parenchyma is doubtful. In the spleens of all three of our cases there was evidence of destruction of some of the follicles, remnants of which surrounded the central arteriole and were compressed by the surrounding myeloid tissue. Although the follicles are destroyed in most cases of leukemic infiltration of the spleen, occasionally in all types of leukemia they are preserved.[40] Callender's[41] Figure 3 depicts a histologically typical lymphoid follicle in the spleen from a case of myelogenous leukemia.

He observed that the myeloid proliferation involved the red pulp, between the lymphoid follicles.

In all of our cases giant cells of megakaryocytic type were noted. In two cases these cells were the elements chiefly responsible for the gross nodulation. Such nodules have been described previously.[4,18,31,32,33,42] Megakaryocytes frequently have been noted in the spleen in myelogenous leukemia.[40,43-46] It does not seem that their presence in excessive numbers in aleukemic myelosis constitutes a valid justification for segregating this disease from leukemia. They represent only one element of a pleomorphic myeloid cellularity. Furthermore, the exaggerated megakaryocytic proliferation is an inconstant feature of aleukemic myelosis. Some cases may exhibit minimal megakaryocytic metaplasia to a degree commonly observed in myelogenous leukemia, as exemplified by case 3.

The significance of the giant cells has been a subject of controversy, as to their relationship with megakaryocytes. The majority of observers regard the cells as megakaryocytes, a view to which we subscribe. Minor alterations in nuclear and cytoplasmic structure, staining reactions, cytoplasmic granulations, etc., in which they differ from the megakaryocytes of normal bone marrow,[42] would scarcely outweigh the fact that the splenic reaction is incontrovertibly an extra-osseous myelosis, and the only giant cells of myeloid tissue are megakaryocytes. Furthermore, minor morphologic variations from the megakaryocytes of normal marrow can be expected if the neoplastic concept of leukemia is accepted.

The alteration in the blood platelets in case 1 permits interesting speculation when correlated with the abnormal megakaryocytic reaction of the spleen. The megakaryocyte is the parent cell of the platelet.[47] One is tempted to associate the abnormal platelets with the abnormal splenic giant cells, as did Carpenter and Flory,[14] a relationship in no manner established in our case. Morphologic alterations in the blood platelets are commonly encountered in leukemia[48] and less frequently in other diseases. Abnormal platelets similar to those seen in case 1 have been described also by Rosenthal and Erf,[37] and Downey and Nordland.[31]

Liver

The gross appearance of the liver in our cases was not particularly striking. Myeloid infiltration was not grossly evident. In case 3 early portal cirrhosis had caused delicate nodulation. Microscopically, the hepatic sinusoids contained myeloid cells in various stages of development, the immature forms predominating. Giant cells presenting megakaryocytic characteristics were numerous. They appeared to take

origin from the Kupffer cells, an observation supported by the presence of transitional stages of development.

The reaction of the liver in myelogeneous leukemia consists of diffuse myeloid infiltration generally involving both the sinusoidal spaces and the portal areas. Giant cells of megakaryocytic type are not unusual. Aleukemic myelosis presents a myeloid infiltration different from typical leukemia in only one significant feature in many of the recorded cases, *i.e.*, absence of portal infiltrations. Such infiltrations were observed in case 3 and serve as one feature, among others, linking the disease with the more typical forms of myelogenous leukemia.

Lymph Nodes

In cases 1 and 2 there was no clinical evidence of lymph node involvement, which was confirmed at autopsy in the latter case. The paravertebral lymph nodes of case 3 were only moderately enlarged. The microscopic picture was one of diffuse myeloid infiltration of the pulp similar to that of the spleen. Such a state of myelosis of the lymph nodes is a common finding in myelogenous leukemia.[44,45,49-51]

Bone Marrow

Under conditions of the average post-mortem examination it is not possible to examine the skeleton thoroughly, and routine procedures generally constitute removal, for microscopic examination, of a portion of femur, vertebral body, sternum or rib, rarely all. That the examination of a single region accurately reveals the general state of the entire hematopoietic marrow is subject to question. Evidence indicates that such uniformity in the marrow cannot always be expected.[2,18,44,52,53] Of interest is Mallory's [54] observation of a case of leukemia, proved by biopsy of bone marrow, which subsequently developed a totally aplastic marrow.

In the field of clinical medicine implicit confidence is frequently placed upon a differential count of cells aspirated from sternal marrow, based upon the belief that comparatively few cells from the sternum reflect the general state of the hematopoietic marrow. This confidence is unshaken by the technical difficulties involved and strengthened by the knowledge that in many cases the procedure has been specifically diagnostic.

The sections of bone marrow from our two autopsy cases were considered to be sufficiently representative to reflect the true state of this tissue. Technically suitable preparations of marrow were available from three or more areas in each case. The reaction in both cases was that of intense myeloid hyperplasia which had resulted in a replace-

ment of much of the marrow fat. Myeloid cells in all stages of development were identified. Moderately frequent mitotic figures were noted in the more immature cells. In both cases megakaryocytes were numerous. The cellular pleomorphism and residual fat content differed from the usual picture of myelogenous leukemia; on the other hand, the intense cellularity, general state of cellular immaturity, the mitotic figures, and the suppression of erythropoietic activity were features to be expected in a leukemic marrow. The diminution of the erythropoietic tissue may be related to the presence of the immature erythrocytes noted in the circulating blood of both cases.

The striking similarity of the clinical, hematologic, and general pathologic features of numerous reported cases of aleukemic myelosis, differing only in respect to the bone marrow, has led to the belief held by many observers, including ourselves, that the marrow reactions are variants within one and the same disease. Jackson, Parker, and Lemon,[29] in presenting 10 cases of "agnogenic myeloid metaplasia," reported on the bone marrow findings as follows: normal marrow, 1 case; hyperplastic marrow, 1 case; aplastic marrow, 1 case; fibrosis of marrow (myelofibrosis), 5 cases; osteosclerosis, 1 case; and 1 case, unknown. "Hyperplastic foci of leukopoiesis"[36] in an otherwise fibrous marrow render interpretation particularly difficult in respect to the existence of hyperplasia or sclerosis.

Osteosclerosis of the type under discussion (excluding the Albers-Schönberg type) depends upon a thickening of the bony lamellae of the spongiosa by deposition of new bone at the periphery. From the numerous descriptions in the literature one gains the impression that the lamellar thickening is always associated with fibrosis of the adjacent marrow and is a manifestation of osseous metaplasia of the fibrous stroma. Conceivably, myelofibrosis and osteosclerosis may represent different stages of the same fundamental process. This interpretation has been expressed by others.[11,12,55]

Osteosclerosis cannot be regarded as a specific entity. It is a reactive lesion of bone marrow resulting from several possible stimuli,[18,56-58] such as secondary carcinoma of osteoplastic type, polycythemia vera, and exogenous toxins, as well as the myelosis of leukemia. Although it is generally recognized that fibrosis of the marrow may occur in myelogenous leukemia, one of the strongest of the criteria serving to differentiate aleukemic myelosis from leukemia has been the presence, in some cases, of osteosclerosis. In view of the intimate and probable causal relationship between myelofibrosis and osteosclerosis, the validity of this criterion is subject to question. However, until the finer structure of the bones has been more extensively studied, the signifi-

cance of osteosclerosis remains uncertain. The occurrence of osteosclerosis in both leukemic and aleukemic myelosis has been reported many times.[11,12,52*,53,58-61]

In the review of the bone marrow of 97 cases of leukemia by Churg and Wachstein,[15] 6 cases were found to show myelofibrosis. One case (case 4) of osteosclerosis was discovered and a revision of diagnosis to a nonleukemic entity followed. The white blood cell count had been as high as 125,000 per cmm., and 10 to 20 per cent myelocytes and 8 to 11 per cent myeloblasts were noted in the blood smears. The liver, spleen, and lymph nodes showed widespread myelosis, and myeloid infiltrations of epicardium, lungs, and kidneys were observed. If 6 cases of leukemia in that series could progress to a stage of myelofibrosis, why might not one case progress to osteosclerosis?

Thus, extreme diversity of the bone marrow may exist in aleukemic myelosis. The transition of normal marrow to either a hyperplastic or hypoplastic type and, in the latter event, to atrophy and fibrosis is readily conceivable. Thus, the diversified alterations of the marrow can be traced, step by step, from one extreme to the other. One extreme is the massive proliferation of typical leukemia; the opposite extreme is myelofibrosis and osteosclerosis.

In view of the fact that most of the reported cases of osteosclerosis have been associated with aleukemic blood, it would seem that a causal relationship may exist between the two. The diminution in the myeloid tissue of the bone marrow coincident with the development of osteosclerosis obviously results in a diminished supply of leukocytes to the blood from this source. The extra-osseous myeloid foci may not contribute immature cells to the blood with the same facility possessed by active bone marrow. Still other unknown factors, however, are responsible for the aleukemic state of the blood inasmuch as cases such as we have reported are aleukemic and yet associated with a hyperplastic, not sclerotic, bone marrow.

Interpretation as a Compensatory Mechanism

In many cases presenting evidence of deficient hematopoietic activity of the bone marrow, the extra-osseous myelosis has been regarded as a compensatory phenomenon.[5,6,12,25,27,32,37,39,62] Such a view seems entirely logical but, upon further analysis, several factors weaken this concept. In the great majority of the recorded cases the cellular reaction which resulted in massive enlargement of the spleen was predominantly leukopoietic. It is exceptional to encounter cases in which

---

* The lesion of the bone marrow of this case was designated "ossifying osteomyelitis" which is strongly presumptive of osteosclerosis.

erythropoiesis has been of any significant extent. A similar situation exists in reference to liver and lymph nodes. It would seem that if extra-osseous hematopoiesis were compensatory to failure of the bone marrow, erythropoietic activity would be a prominent feature of the reaction. Much more severe and persistent anemias, of other types, are not productive of the massive extramedullary hematopoiesis found in aleukemic myelosis.

At times the interpretation of deficient function of the bone marrow has been based entirely upon an unjustifiable inference. Ballin and Morse,[27] under the title "Myelophthisic Splenomegaly," reported two cases which we consider identical with ours. The pathologic study was limited to the surgically removed spleens and their interpretation that the splenic reaction was compensatory to bone marrow failure was not supported by any recorded observations of the bone marrow. The visceral myelosis of our cases cannot be regarded as compensatory inasmuch as the bone marrow was overactive. In all other essential respects our cases appear identical with cases having hypoplastic or sclerotic bone marrow. Thus, it would seem that there is no scientific basis for the assumption that the visceral myelosis of this disease is of compensatory nature.

Visceral Infiltrations

The absence of leukemic infiltration in viscera other than the spleen, liver, and lymph nodes in aleukemic myelosis has been a factor in the nonleukemic interpretation. In myelogenous leukemia such infiltrations are variable and inconstant. In an analysis of 123 cases of leukemia by Kirshbaum and Preuss,[51] the kidney was found to be the organ most commonly involved by leukemic infiltrations, excluding spleen, liver, and lymph nodes. In their series, however, the kidney showed no involvement in over one-third of the cases. Thus, the occurrence of visceral infiltrations would appear to be a totally unreliable criterion by which to differentiate aleukemic myelosis from leukemia, and an explanation for their absence will be offered subsequently.

HEMATOLOGIC FEATURES

That the blood picture in myelogenous leukemia is subject to considerable variability is well known, but the extent of the variability possible in any individual case is not entirely clear. The designation aleukemic frequently is applied to cases of leukemia in which the total white cell count of the circulating blood is within normal limits, although examination of a stained blood smear would reveal immature cells. Such observations are not rare, occurring either at one time or another in the natural course of the disease or as a result of therapy.

We consider it advisable to apply the term aleukemic myelosis to cases of myelogenous leukemia which are aleukemic in respect to both the differential count and the total leukocyte count. The basic leukemic nature of such cases is often unrecognized.

It has been questioned whether cases of myelogenous leukemia exist in which adequate repeated studies of stained blood smears by a qualified observer fail to reveal the leukemic proliferation which exists in the myeloid tissue of the body.[63] Since it is generally accepted that there are cases of myelogenous leukemia in which there is relatively slight alteration in the blood picture, the assumption that cases do not exist without any characteristic alteration of the white blood cells seems overly dogmatic.

Of interest are the blood studies of our three cases, two of which are presented with the implication that genuinely and permanently aleukemic forms of the disease do exist. In case 1, the preoperative blood examinations gave no evidence of a leukemic process. The postoperative studies at first merely aroused a suspicion, but a frankly leukemic picture subsequently developed. This was of major importance in the reversal of our original nonleukemic interpretation of the splenic reaction. In cases 2 and 3, adequate blood examinations over a period of several years failed to establish the diagnosis of leukemia.

Perhaps the most important factors leading to a nonleukemic interpretation of aleukemic myelosis have been the normal or only moderately elevated number of leukocytes in the blood, and their state of relative maturity. Such factors, in themselves, do not exclude leukemia. In reference to the total number of the white blood cells, it is universally accepted that myelogenous leukemia may exist without a numerical increase.[64] In 5 of 53 cases of myelogenous leukemia reported by Kirshbaum and Preuss [51] the total white count fell to less than 4,000 per cmm. In reference to cellular maturity, we have encountered cases of chronic myelogenous leukemia in which blast forms were not demonstrable, myelocytes being the least mature cell type present. Such cases are not unusual.[50] When depressed total count and cellular maturity are combined, the leukemic nature may not be recognized.

It is possible that many of the mature leukocytes of the circulating blood in cases such as ours are actually well differentiated leukemic cells, differentiated to such a degree that their leukemic nature cannot be recognized morphologically. Myelogenous leukemia of highly differentiated character may produce structural abnormalities only in the parent myeloid tissue and individual cellular elements of such growth, the circulating leukocytes, may be cytologically normal or negligibly

altered. Somewhat analogous are the individual neoplastic cells of a well differentiated adenocarcinoma, the diagnosis of which may be dependent entirely upon atypical organoid structure rather than upon morphologic alteration of individual tumor cells.

Much attention has been centered upon the presence of immature cells of the erythrocytic series. The presence of these, associated with anemia and immature granulocytic forms, has led to the designation "leuko-erythroblastic anemia,"[23] a descriptive term of hematologic significance without pathologic implication. It is pertinent to emphasize that young erythrocytic cells occur almost constantly in the circulating blood of cases typically leukemic,[1,31,41,45,50] so their presence in aleukemic myelosis does not serve to differentiate it from leukemia. When a blood smear is flooded with immature myelogenous cells the nucleated red cells are much less conspicuous than they are in an aleukemic blood. The immature granulocytic cells in the blood of many cases of "leuko-erythroblastic" anemia (Vaughan type[23]), regardless of their number or relative maturity, are a manifestation of the underlying myeloid proliferation which we regard as leukemic. The state of the peripheral blood is not necessarily an indication of the condition of the somatic myeloid tissues and such blood pictures cannot exclude an underlying leukemic process. To characterize a disease as an entity based upon the blood picture and to misconstrue the significance of the underlying pathologic process leads to interpretive and nosologic confusion.

In many reported cases the authors' interpretations of the immature myeloid cells of the blood are not entirely clear. Thus, Downey, Palmer, and Powell,[17] in recording the differential blood count in a case of "atypical myelosis," did not include those cells in their differential percentages which, under "remarks," they designated as "many myeloblasts." Throughout the literature of this disease one encounters strikingly high percentages of immature myeloid cells in the blood, often associated with considerable elevation in the total white count. In Table III are several examples. The list could be extended considerably.

All of the cases included in Table III were presented by the authors, either by statement or implication, as examples of a disease distinct from leukemia. In the literature of this disorder the highest total count that we have encountered in cases presented by the author as representing a disease other than leukemia was 227,000 (Rosenthal and Erf,[37] case 14). In this case 42 per cent of the leukocytes were myelocytes. The authors excluded leukemia because of fibrosis of the bone marrow. In view of the not infrequent occurrence of myelofibrosis in leukemia,[15] one is justified in questioning this interpretation.

TABLE III

Total and Differential White Blood Cell Counts in Cases Considered by the Respective Authors as Examples of Diseases Differing from Leukemia

Author	Total white blood cells	Myelocytes (per cent)	Premyelocytes (per cent)	Myeloblasts (per cent)	Additional data (per cent)
Rosenthal and Erf[37] (case 6)	124,000	10		3	
Jordan and Scott[39]	32,000	20	25		Basophils 6
Vaughan and Harrison[38]	49,000	3		9	Basophils 7 Eosinophils 21
Hickling[13] (case 7)	60,000	20 (and blasts)			
Reich and Rumsey[30] (case 5)	86,000	20		6	
Carpenter and Flory[14]	86,300	11		8	
Downey and Nordland[31]	79,700	1	10	24	

(When more than one observation was recorded, the figures in the above table represent the highest.)

CLINICAL FEATURES

The prolonged clinical course of many cases of aleukemic myelosis, as exemplified by our second case with a history of splenomegaly for 19 years, has been advanced as a feature differentiating the disorder from leukemia. Admittedly, such a survival period in leukemia is most unusual but scarcely serves as a scientific basis for its exclusion. Forkner[45] observed a patient with chronic myelogenous leukemia for 18 years. Survival for a period of 25 years has been reported.[65]

Prolonged survival periods in aleukemic myelosis are unusual and the survival time of many cases is fully equivalent to that of the usual case of myelogenous leukemia. Körner[66] reported a case, which was indistinguishable pathologically from aleukemic myelosis, with an accelerated clinical course and a rapidly fatal outcome. Zypkin[10] described acute myelogenous leukemia, aleukemic in reference to the blood smear, many years ago. Baldridge and

Fowler [7] observed a more rapidly fatal course in the aleukemic type of myelosis as compared with the leukemic forms. Thus, it would seem that there is great variability in the duration of the disease, just as there is in typical myelogenous leukemia.

It has been noted that the majority of patients with aleukemic myelosis, treated by irradiation, do not show the beneficial response characteristic of myelogenous leukemia and many have apparently suffered from such therapy. The beneficial response in leukemia is generally reflected by a drop in the total white count, and it is a well known observation that radiotherapy in leukemia is hazardous when the total white count of the blood drops below a level somewhat greater than the normal. Thus, one might predict an indifferent or unfavorable response in cases of leukemia with an initial normal or only slightly elevated total white count. That all cases may not exhibit such an unfavorable response is indicated by the observation of Dameshek [57] of a case presenting all the diagnostic criteria of "agnogenic myeloid metaplasia" in which the patient improved following radiotherapy. Benefit from radiotherapy has been reported by others [3,6,24,28,67] The response to radiotherapy does not serve as a valid criterion for distinguishing the disorder from leukemia.

A large percentage of the recorded cases in which the patients were subjected to splenectomy terminated fatally within a short period of time following the operation. Patients surviving for several years following splenectomy have been observed. As a practical consideration in the therapy of such cases the danger of splenectomy is evident, but as a factor differentiating the disease from myelogenous leukemia it is valueless. No beneficial effect can be expected from splenectomy in myelogenous leukemia.

INTERMEDIARY FORMS

To establish the leukemic nature of the disease under discussion, certain cases are presented which constitute intermediary forms linking this disorder with typical myelogenous leukemia. Case 3 serves as one example in which portal infiltrations of the liver were observed. Levy's [2] case, reported as leukemia, showed myelosis of the liver and spleen with retention of the lymphoid follicles, leukemic infiltrations of the kidneys, and hyperplasia of the bone marrow. The total white blood cell count had been normal. Mettier and Rusk [36] reported a case of leukemia (case 1) with a normal total white blood cell count. The spleen, removed surgically, showed myeloid metaplasia with lymphoid follicles normal in size and number. The white blood cell count exceeded 25,000 per cmm. postoperatively, with 54 per cent myelocytes

in the blood smear. At autopsy the bone marrow was grossly osteo-sclerotic, microscopically fibrotic. The lymph nodes showed extensive myelosis. Case 2, presented as leukemia by the same authors, showed clinical features of chronic myelogenous leukemia. Pathologically, the essential structure of the spleen was preserved. There was no portal infiltration of the liver but numerous myeloid cells were present in the sinuses. Small myeloid infiltrations of the testes were noted. The bones showed myelofibrosis. A case reported by Krumbhaar and Stengel [40] as chronic myelogenous leukemia of mild myelopoietic ability showed no significant elevation of the white blood cell count but many immature myeloid cells in the blood smear. The bone marrow was hyperplastic. The liver showed no myeloid reaction. Myelocytic infiltration of the splenic pulp had not destroyed the lymph follicles. Körner's [06] case of myelogenous leukemia had a normal total white blood cell count and immature myeloid cells in the blood smear. Death occurred a few weeks after the onset of symptoms. Autopsy showed myelosis of spleen, liver, and lymph nodes with numerous giant cells of megakaryocytic type. The bone marrow was hyperplastic. Hickling's [13] case presented splenomegaly for years, and hemorrhagic phenomena before death. There was myeloid metaplasia of the spleen with destruction of the lymphoid follicles, myelosis of the liver, and myeloid infiltration of the kidney and skin. The bone marrow appeared normal.

NATURE OF ALEUKEMIC MYELOSIS

It is a well known fact that in embryonic development hematopoietic tissue normally occurs in extra-osseous locations, notably in the spleen and liver. When the function of blood formation is acquired by the bone marrow of the fetus there is a regression and disappearance of the extra-osseous foci which reappear in later life only under pathologic conditions. The return of hematopoietic activity to the spleen, liver, and lymph nodes under conditions in which it is obviously compensatory to blood loss, destruction, or a maturation deficiency, is sufficient grounds for the assumption that constant potentiality exists in these extra-osseous tissues to revert to hematopoiesis. As far as is known, only one common functional and anatomic relationship exists between bone marrow, liver, spleen, and lymph nodes: the reticulo-endothelial system. Although controversial, it seems likely that it is the proliferation and differentiation of the reticulo-endothelial cells that produces hematopoietic tissue in the extra-osseous localities under certain pathologic conditions.

It has not been fully established whether the extra-osseous myelosis in leukemia is a manifestation of local origin or the result of coloniza-

tion (metastasis).[68,69] The myeloid infiltration of viscera, such as kidney and lung, which do not possess an intrinsic reticulo-endothelial system,* can be explained only by the assumption that such foci are the result of continuous proliferation of immature myeloid cells arriving in these viscera through the blood stream. However, such an interpretation for the myelosis of organs possessing a reticulo-endothelial system does not necessarily obtain and our observations refute such an *exclusive* interpretation.

In our cases the local origin of the myeloid cells in the spleen, liver, and lymph nodes appeared to be well established both by direct histologic features and indirectly by inference. Histologically, the development of megakaryocytes and myeloblasts from the reticulo-endothelial cells was observed through numerous transitional forms. Inferentially, the absence of immature myeloid cells from the blood seemed to preclude their appearance in spleen, liver, and lymph nodes by a process of leukemic colonization. This is particularly significant in reference to the megakaryocytes. Although such cells have been described in the blood,[70] they represent fixed cells not prone to be widely disseminated by the vascular system. Furthermore, the occurrence of an occasional megakaryocyte in the circulating blood would scarcely explain the diffuse megakaryocytic reaction of the extra-osseous myelosis exhibited in our cases.

It has been claimed that the failure to demonstrate immature leukocytes in the blood does not exclude a metastatic process in leukemia. An analogy is drawn with extensive carcinomatosis in which tumor cells cannot be demonstrated in the blood.[44] The analogy is not fully valid. Theoretically, each metastatic tumor in carcinomatosis may represent vascular dissemination of only one tumor cell which gives rise to a large secondary growth through continuous proliferation. The most extensive carcinomatosis represents vascular dissemination of only a relatively few tumor cells and their detection in a blood smear would be a fortuitous rarity. In myelogenous leukemia, the diffuse character of the leukemic infiltrations suggests that the majority of the cells are individually metastatic. These infiltrations do not represent, as in carcinomatosis, a solid compact tumor mass having origin from a single metastatic cell. Hence, the absence of immature myeloid cells in the blood stream of cases such as we have reported is indirect evidence supporting the theory of local origin of the myelosis. Furthermore, the metastatic concept does not adequately explain the invariably predominant distribution of the myelosis to liver, spleen, and lymph nodes.

---

* Distinction is made between the sinusoidal endothelium of the reticulo-endothelial system and the interstitial mesenchymal reticulum cells. The above comment is in reference to the former.

The occurrence of myeloid metaplasia in spleen and elsewhere in diseases unrelated to leukemia, in which the factor of colonization or metastasis is not an issue, offers strengthening evidence for local origin. The observations of many investigators support this concept.[17,28,50,66,71-76]

It appears likely that aleukemic myelosis is the expression of some obscure stimulus acting upon the reticulo-endothelial system, resulting in abnormal proliferation and differentiation of reticulo-endothelial cells into myeloid cells. It appears probable that the abnormal growth stimulus is the same as that operating in myelogenous leukemia, but quantitatively altered in this disorder, generally resulting in a myeloid proliferation of reduced intensity, of more prolonged course, and productive of a more diversified and more highly differentiated cellularity. Under this abnormal stimulus the reticulo-endothelial cells are capable of differentiating into either megakaryocytes or granulocytic cells, some of which may escape into the circulating blood. Their relative maturity serves to explain the absence of general leukemic infiltrations of viscera. Such cells do not inherit the same degree of growth potentiality as the more anaplastic cells which characterize the usual case of leukemia in which visceral infiltrations of organs not possessing an intrinsic reticulo-endothelial system can be explained only on the basis of colonization. Capability of reproduction does not seem to be possessed by myeloid cells at stages of development beyond the myeloblast. The capability of reticulo-endothelial cells under either normal or pathologic conditions to differentiate into megakaryocytes and myeloid cells has been described previously.[42,69,72,74,75]

The scope of such a concept obviously encompasses the myelosis of leukemia generally, in which the entire reticulo-endothelial system responds as a unit to the obscure leukemic stimulus. With such an interpretation, much of the myeloid reaction in organs possessing a reticulo-endothelial system, such as spleen and liver, may represent generation *in situ* and not a colonizing process. This would modify the interpretation that myelogenous leukemia is a disorder predominantly or even primarily of bone marrow. Such a concept is not new [1,10,44,50] but it has not received wide acceptance.

Incidence. The apparent rarity of genuinely aleukemic myelogenous leukemia (aleukemic myelosis) appears to be due, largely, to a misconception of the clinical, hematologic, and pathologic findings. When one incorporates with the leukemias the numerous cases of the type that we have reported and which are described under the many nonleukemic designations, the incidence is materially increased. A recent review [37] of the literature in reference to sclerosis of bone of the type associated

with aleukemic myelosis recorded 75 cases. A considerable number of cases of aleukemic myelosis without osteosclerosis appear in the literature. Consideration of these factors leads one to conclude that the disease is not rare. This is consistent with the experience of Baldridge and Fowler [7] who reported a permanently aleukemic state in approximately 5 per cent of their cases of diffuse myelosis (myelogenous leukemia). Incomplete data, individualistic nomenclature, the inclusion of cases of aleukemic myelosis in series with unrelated diseases, and the difficulty of formulating exact criteria to classify the numerous cases intermediary between aleukemic myelosis and chronic myelogenous leukemia render a complete and accurate compilation of recorded cases impossible, and this we have not attempted.

Diagnosis. Familiarity with the clinical features of aleukemic myelosis is one of the most important factors in the clinical diagnosis of the disease. Of foremost importance in the differential diagnosis are the diseases causing splenic enlargement. In many cases the presence in blood smears of granular leukocytes at various stages of immaturity, unexplainable by any infectious process, is presumptively diagnostic. It is important to stress the fact that an absence of immature leukocytes in the blood smear does not exclude aleukemic myelosis. Bone marrow studies may be diagnostic in those cases with myeloid hyperplasia; the great variability in the structure of the bone marrow, however, makes this procedure unreliable. In those cases with osteosclerosis, radiologic study of the bones will be strongly confirmatory. Diagnosis by splenic puncture is possible [1,2,28] but the results may be equivocal and the procedure is not without danger. In some cases the nature of the disease may be apparent only from autopsy study.

Treatment. In the majority of the reported cases the response to irradiation has been either indifferent or unfavorable. If radiotherapy is employed, the treatment should not be vigorous and the patient should be under careful observation. Splenectomy is contraindicated. Other therapeutic measures are merely symptomatic and supportive.

SUMMARY AND CONCLUSIONS

1. A study of three exemplary cases, with a review of the appropriate literature, has led to the interpretation of a syndrome characterized by splenomegaly and nonspecific alterations in the blood as a form of myelogenous leukemia. Many cases of this type are genuinely aleukemic.

2. Although this disorder is not particularly rare, its leukemic nature has not been generally recognized because of atypical clinical, hematologic, and pathologic features. Its identity has been obscured

by a diversified terminology. Aleukemic myelosis is an appropriate designation.

3. Osteosclerosis may occur in the course of myelogenous leukemia. Its occurrence favors the development of an aleukemic state of the blood.

4. The criteria alleged to differentiate the disorder from leukemia do not withstand critical analysis.

5. Cases of the type reported present strong evidence to support the belief that much of the myeloid reaction of spleen, liver, and lymph nodes in myelogenous leukemia is not an expression of colonization (metastasis) but of myeloid transmutation of the local reticulo-endothelial system of these organs.

REFERENCES

1. Hirschfeld, H. Die generalisierte aleukämische Myelose und ihre Stellung im System der leukämischen Erkrankungen. *Ztschr. f. klin. Med.*, 1914, 80, 126–173.
2. Levy, M. Zur Diagnose der aleukämischen Myelose nebst kurzen Bemerkungen über Therapie und Verlauf. *Folia haemat.*, 1919–20, 25, 63–70.
3. Jores, A. Ein Fall von aleukämischer Myelose mit Osteosklerose des gesamten Skelettsystems. *Virchows Arch. f. path. Anat.*, 1927, 265, 845–851.
4. Jaffé, R. H. Aleukemic myelosis. *Arch. Path.*, 1927, 3, 56–72.
5. Mavros, A. Aleukämische, besser "nichtleukämische" Myelose mit Osteosklerose. *Folia haemat.*, 1930–31, 43, 323–339.
6. Stephens, D. J., and Bredeck, J. F. Aleukemic myelosis with osteosclerosis. *Ann. Int. Med.*, 1932–33, 6, 1087–1096.
7. Baldridge, C. W., and Fowler, W. M. Aleukemic myelosis. *Arch. Int. Med.*, 1933, 52, 852–876.
8. Pinkerton, H. Aleukemic leukemia and atypical leukemoid conditions. Report of 7 cases, including one of acute erythroblastosis. *Arch. Path.*, 1929, 7, 567–600.
9. Hynes, M. Aleukaemic leukaemia. *Quart. J. Med.*, 1940, 9, 177–192.
10. Zypkin, S. M. Über die akute Pseudoleukämie und die Wechselbeziehungen zwischen den Blutkrankheiten. *Virchows Arch. f. path. Anat.*, 1912, 209, 56–96.
11. Zypkin, S. M. Ein Fall von lymphatischer Pseudoleukämie mit Osteosklerose und Ausgang in akute lymphatische Leukämie. *Folia haemat.*, 1927–28, 35, 7–20.
12. Wolf, C. Über einen Fall von osteosklerotischer Pseudoleukämie. Beitrag zur Frage der Osteosklerosen. *Beitr. z. path. Anat. u. z. allg. Path.*, 1932, 89, 151–182.
13. Hickling, R. A. Chronic non-leukaemic myelosis. *Quart. J. Med.*, 1937, 6, 253–275.
14. Carpenter, G., and Flory, C. M. Chronic nonleukemic myelosis. Report of a case with megakaryocytic myeloid splenomegaly, leuko-erythroblastic anemia, generalized osteosclerosis and myelofibrosis. *Arch. Int. Med.*, 1941, 67, 489–508.

15. Churg, J., and Wachstein, M. Osteosclerosis, myelofibrosis and leukemia. *Am. J. M. Sc.*, 1944, 207, 141–152.

16. Helly, K. Leukämien. In: Henke, F., and Lubarsch, O. Handbuch der speziellen pathologischen Anatomie und Histologie. J. Springer, Berlin, 1927, 1, Pt. 2, 1015–1099.

17. Downey, H., Palmer, M., and Powell, L. The origin of the megakaryocytes in the spleen and liver in a case of atypical myelosis. *Folia haemat.*, 1930, 41, 55–72.

18. Hewer, T. F. Megakaryocytic myelosis with osteosclerosis. *J. Path. & Bact.*, 1937, 45, 383–390.

19. Weber, F. P. Leukanaemia. *Lancet*, 1904, 1, 1503.

20. Bushnell, F. G., and Hall, D. G. Leukanaemia. *Edinburgh M. J.*, 1906, 19, 339–343.

21. Symmers, D. Leukanemia. *J. A. M. A.*, 1921, 76, 156–158.

22. Vaquez, H., and Aubertin, C. Nature de l'anémie splénique myéloïde. *Compt. rend. Soc. de biol.*, 1904, 56, 792–794.

23. Vaughan, J. M. Leuco-erythroblastic anaemia. *J. Path. & Bact.*, 1936, 42, 541–564.

24. McMichael, J., and McNee, J. W. Leuco-erythroblastosis. *Edinburgh M. J.*, 1936, 43, 303–314.

25. Chapman, E. M. Osteosclerotic anemia. *Am. J. M. Sc.*, 1933, 185, 171–177.

26. Weil, P. E., and Clerc, A. Note sur la splénomégalie avec anémie et myélémie. *Compt. rend. Soc. de biol.*, 1904, 56, 945–946.

27. Ballin, M., and Morse, P. F. Myelophthisic splenomegaly. *J. A. M. A.*, 1927, 89, 1671–1672.

28. Carnot, P., Caroli, J., and Busson, A. La myélose hépato-splénique aleucémique. Sur diagnostic par la ponction de la rate. *Paris méd.*, 1935, 95, 449–455.

29. Jackson, H., Jr., Parker, F., Jr., and Lemon, H. M. Agnogenic myeloid metaplasia of the spleen. A syndrome simulating other more definite hematologic disorders. *New England J. Med.*, 1940, 222, 985–994.

30. Reich, C., and Rumsey, W., Jr. Agnogenic myeloid metaplasia of the spleen. Report of five cases illustrating diagnostic difficulties and the danger of splenectomy and radiation therapy. *J. A. M. A.*, 1942, 118, 1200–1204.

31. Downey, H., and Nordland, M. Hematologic and histologic study of a case of myeloid megakaryocytic hepato-splenomegaly. *Folia haemat.*, 1939, 62, 1–39.

32. Donhauser, J. L. The human spleen as an haematoplastic organ, as exemplified in a case of splenomegaly with sclerosis of the bone-marrow. *J. Exper. Med.*, 1908, 10, 559–574.

33. Tudhope, G. R. Splenomegaly with myeloid transformation. *J. Path. & Bact.*, 1937, 44, 99–102.

34. Watson, C. J. A peculiar case of splenomegaly with anemia. *Arch. Path.*, 1926, 1, 654–658.

35. Rathery, F. Splénomégalie du type myéloïde sans myélocythémie. *Compt. rend. Soc. de biol.*, 1902, 54, 138–140.

36. Mettier, S. R., and Rusk, G. Y. Fibrosis of the bone marrow (myelofibrosis) associated with a leukemoid blood picture. Report of two cases. *Am. J. Path.*, 1937, 13, 377–388.

37. Rosenthal, N., and Erf, L. A. Clinical observations on osteopetrosis and myelofibrosis. *Arch. Int. Med.*, 1943, 71, 793–813.

38. Vaughan, J. M., and Harrison, C. V. Leuco-erythroblastic anaemia and myelosclerosis. *J. Path. & Bact.*, 1939, 48, 339–352.

39. Jordan, H. E., and Scott, J. K. A case of osteosclerosis with extensive extramedullary hemopoiesis and a leukemic blood reaction. *Arch. Path.*, 1941, 32, 895–909.

40. Krumbhaar, E. B., and Stengel, A. The spleen in the leukemias. *Arch. Path.*, 1942, 34, 117–132.
41. Callender, G. R. Tumors and tumor-like conditions of the lymphocyte, the myelocyte, the erythrocyte and the reticulum cell. *Am. J. Path.*, 1934, 10, 443–465.
42. Barth, H. Über Riesenzellbildungen bei Leukämie. ("Leukämische Endotheliose.") *Virchows Arch. f. path. Anat.*, 1925, 256, 693–704.
43. Watson, C. J. Lymphosarcoma and Leucosarcoma. In: Downey, H. (ed.) Handbook of Hematology. Paul B. Hoeber, Inc., New York, 1938, 4, 3051–3106.
44. Richter, M. N. Leucemia. In: Downey, H. (ed.) Handbook of Hematology. Paul B. Hoeber, Inc., New York, 1938, 4, 2887–3035.
45. Forkner, C. E. Leukemia and Allied Disorders. The Macmillan Co., New York, 1938.
46. Klemperer, P. The Spleen. In: Downey, H. (ed.) Handbook of Hematology. Paul B. Hoeber, Inc., New York, 1938, 3, 1591–1754.
47. Wright, J. H. The origin and nature of the blood plates. *Boston M. & S. J.*, 1906, 154, 643–645.
48. Olef, I. The differential platelet count. Its clinical significance. *Arch. Int. Med.*, 1936, 57, 1163–1185.
49. Klemperer, P. Giant cell leukemia. *Arch. Path.*, 1934, 18, 286–287.
50. Naegeli, O. Blutkrankheiten und Blutdiagnostik. J. Springer, Berlin, 1931, ed. 5.
51. Kirshbaum, J. D., and Preuss, F. S. Leukemia. A clinical and pathologic study of 123 fatal cases in a series of 14,400 necropsies. *Arch. Int. Med.*, 1943, 71, 777–792.
52. Krumbhaar, E. B. Leukemoid blood pictures in various clinical conditions. *Am. J. M. Sc.*, 1926, 172, 519–533.
53. Schmorl, G. Leukämie mit Ausgang in Osteosklerose. *München med. Wchnschr.*, 1904, 51, 537.
54. Mallory, T. B. Cabot case no. 21281. *New England J. Med.*, 1935, 213, 67–71.
55. Klemperer, P. Discussion of paper by Jacobson.[60]
56. Hirsch, E. F. Generalized osteosclerosis with chronic polycythemia vera. *Arch. Path.*, 1935, 19, 91–97.
57. Dameshek, W. Medical progress; hematology. *New England J. Med.*, 1945, 232, 280–286.
58. Askanazy, M. Knochenmark. In: Henke, F., and Lubarsch, O. Handbuch der speziellen pathologischen Anatomie und Histologie. J. Springer, Berlin, 1927, 1, Pt. 2, 891–901.
59. Heuck, G. Zwei Fälle von Leukämie mit eigenthümlichem Blut- resp. Knochenmarksbefund. *Virchows Arch. f. path. Anat.*, 1879, 78, 475–496.
60. Jacobson, S. A. Myeloid leukemia with osteosclerosis. *Arch. Path.*, 1933, 15, 602–604.
61. Goldzieher, M. A. Discussion of paper by Jacobson.[60]
62. Taylor, H. E., and Smith, R. P. Marrow sclerosis associated with massive myeloid splenomegaly. *Arch. Path.*, 1941, 31, 803–810.
63. Schridde, H. Die blutbereitenden Organe. In: Aschoff L. Pathologische Anatomie. G. Fischer, Jena, 1923, ed. 6, 2, 102. (Cited by Richter.[44])
64. King, J. T., Jr. Aleucocythaemic leukaemia. 1. Acute myeloblastic leukaemia. 2. Chloroma(?). 3. Chronic lymphatic leukaemia. *Bull. Johns Hopkins Hosp.*, 1917, 28, 114–120.
65. Kohn. Cited by Naegeli.[50]
66. Körner, K. Auffallende Riesenzellenbefunde bei akuter Myeloblastenleukämie. *Virchows Arch. f. path. Anat.*, 1926, 259, 617–627.

67. Goldschmid, E., and Isaac, S. Endothelhyperplasie als Systemerkrankung des hämatopoetischen Apparates. *Deutsches Arch. f. klin. Med.,* 1921–22, 138, 291–308.

68. Ewing, J. Neoplastic Diseases. W. B. Saunders Co., New York, 1940, ed. 4, p. 393.

69. Schiller, W. Local myelopoiesis in myeloid leukemia. *Am. J. Path.,* 1943, 19, 809–837.

70. Minot, G. R. Megacaryocytes in the peripheral circulation. *J. Exper. Med.,* 1922, 36, 1–7.

71. Richter, M. N. Discussion of paper by Klemperer.[49]

72. Bloom, W. Myelopoietic Potency of Fixed Cells of the Rabbit Liver. Libman Anniversary Volumes, International Press, New York, 1932, 1, 199–207.

73. Haymaker, W. Metaplasia in the lymph nodes and spleen in a case of myelogenous leukemia. *Bull. Ayer Clin. Lab., Pennsylvania Hosp.,* 1930, no. 12, pp. 55–62. Abstract in: *Arch. Path.,* 1931, 11, 290.

74. Hueper, W. C. Megakaryocytosis in white mice with spontaneous mammary carcinomas. *Am. J. M. Sc.,* 1934, 188, 41–49.

75. Lang, F. J. Myeloid Metaplasia. In: Downey, H. (ed.) Handbook of Hematology. Paul B. Hoeber, Inc., New York, 1938, 3, 2105–2144.

76. Plaut, A. Discussion of paper by Klemperer.[49]

DESCRIPTION OF PLATES

PLATE 50

Fig. 1. Case 1. Spleen. The cut surface is fleshy and studded with small dark nodules.

Fig. 2. Case 1. Spleen. A myeloid nodule containing numerous giant cells occupies the upper half of the field; compressed parenchyma appears below. × 120.

Fig. 3. Case 1. Blood smear showing giant platelet. × 1250.

1

|⊢————— 10 cms. ————————⊣|

2
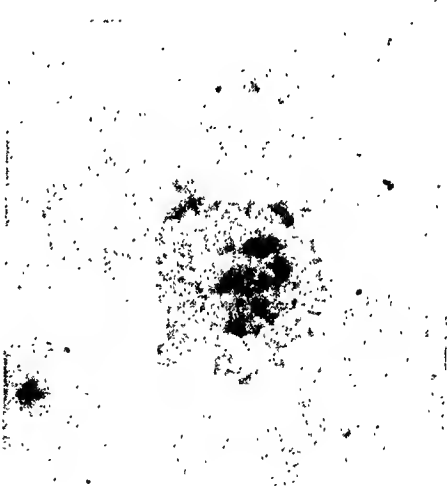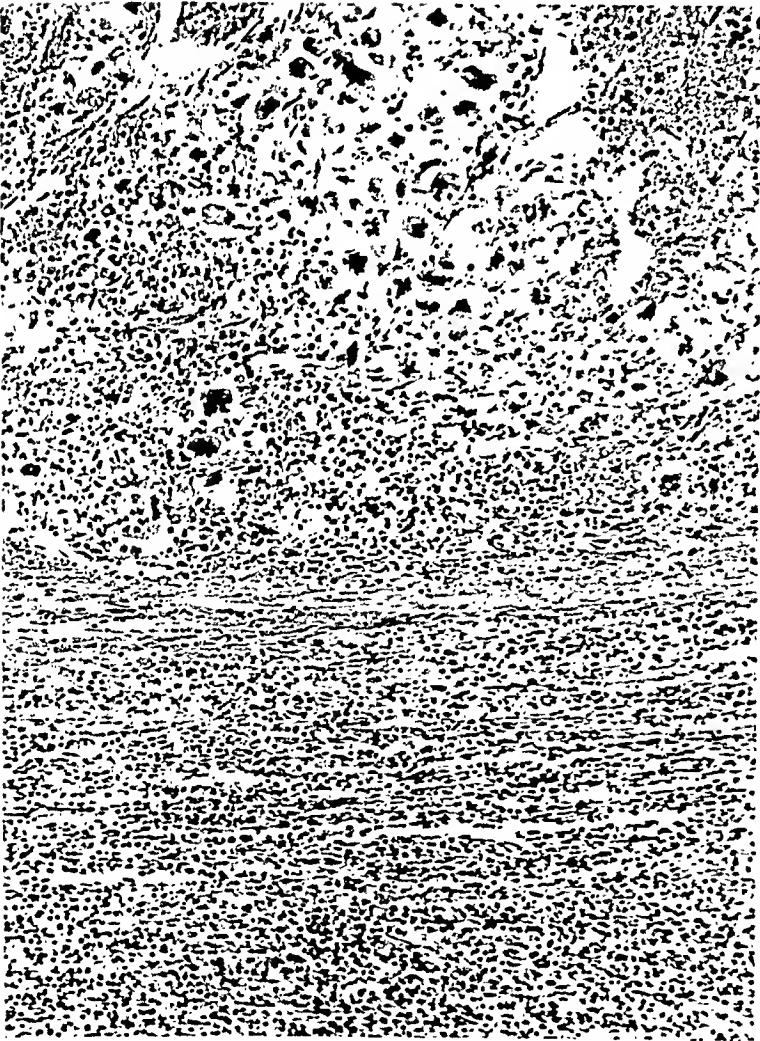
Heller, Lewisohn, and Palin

Aleukemic Myelosis

FIG. 4. Case 2. Spleen. A lymphoid follicle is surrounded by myeloid tissue containing many giant cells of megakaryocytic type. $\times$ 300.

FIG. 5. Case 2. Spleen. Development of myeloblasts from reticulo-endothelial cells within the lumen of a sinus. Of note is the continuity of the myeloblasts with transitional forms of endothelium. $\times$ 650.

4
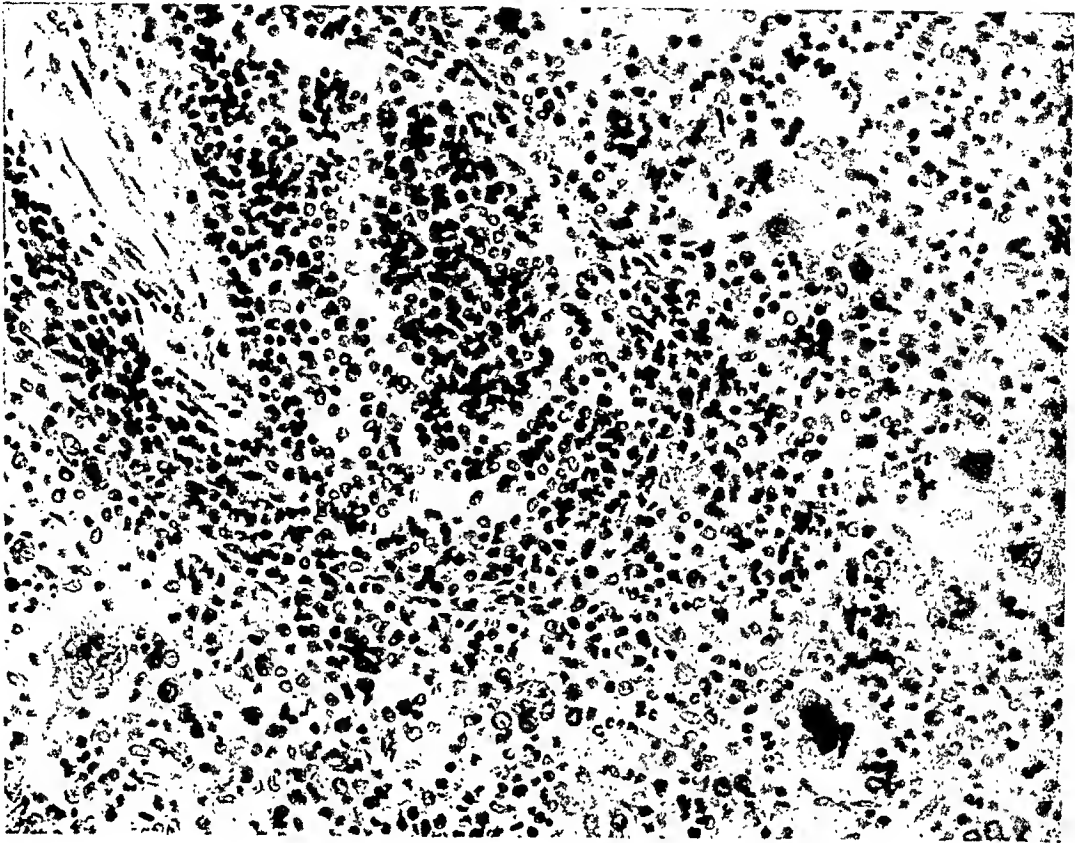
5
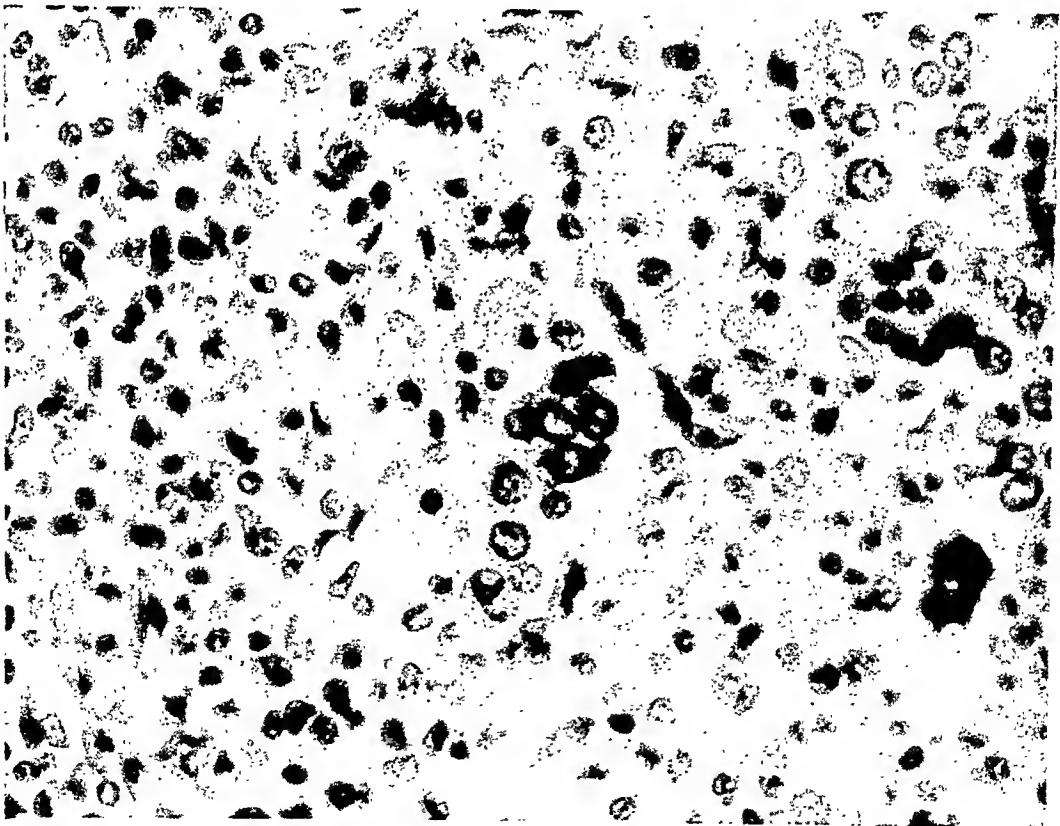
PLATE 52

FIG. 6. Case 2. Spleen. Development of megakaryocytes from reticulo-endothelial cells. In the left upper area of the sinus there is an elongated tri-nucleated megakaryocyte. This cell represents a transitional form from the sinus endothelium. × 650.

FIG. 7. Case 2. Bone marrow, showing myeloid hyperplasia of pleomorphic type with numerous megakaryocytes. × 300.
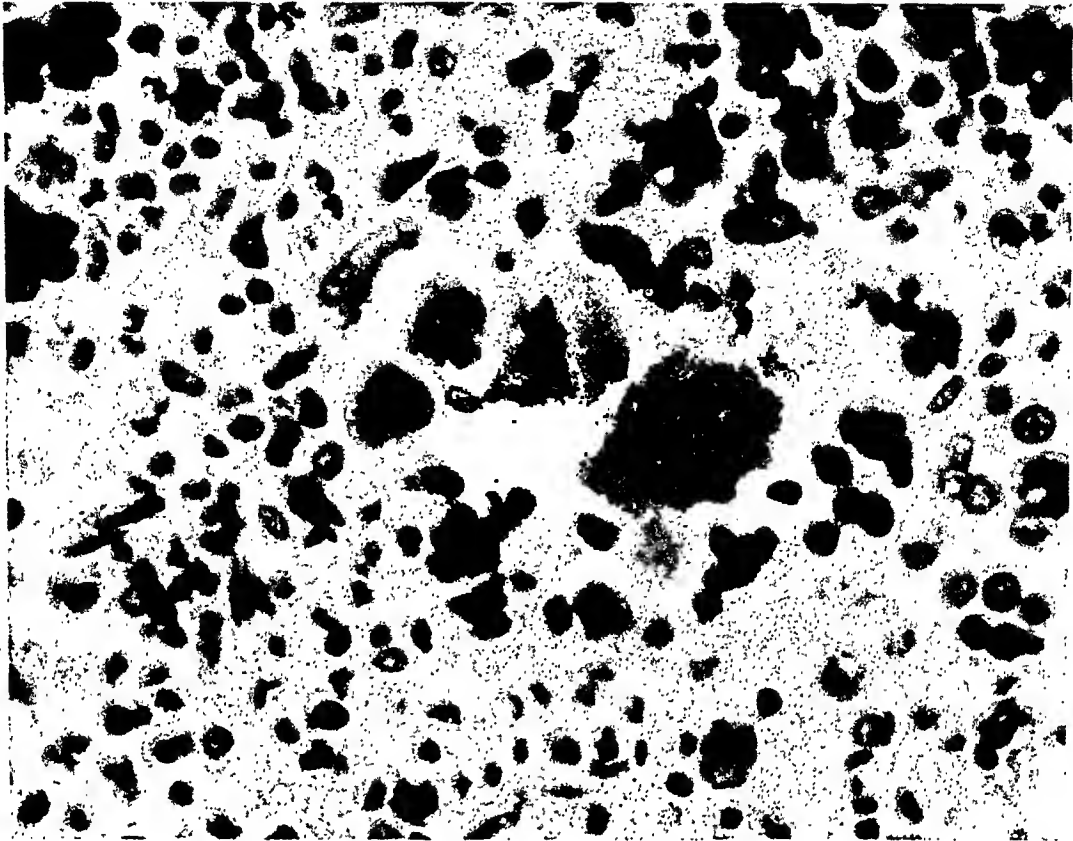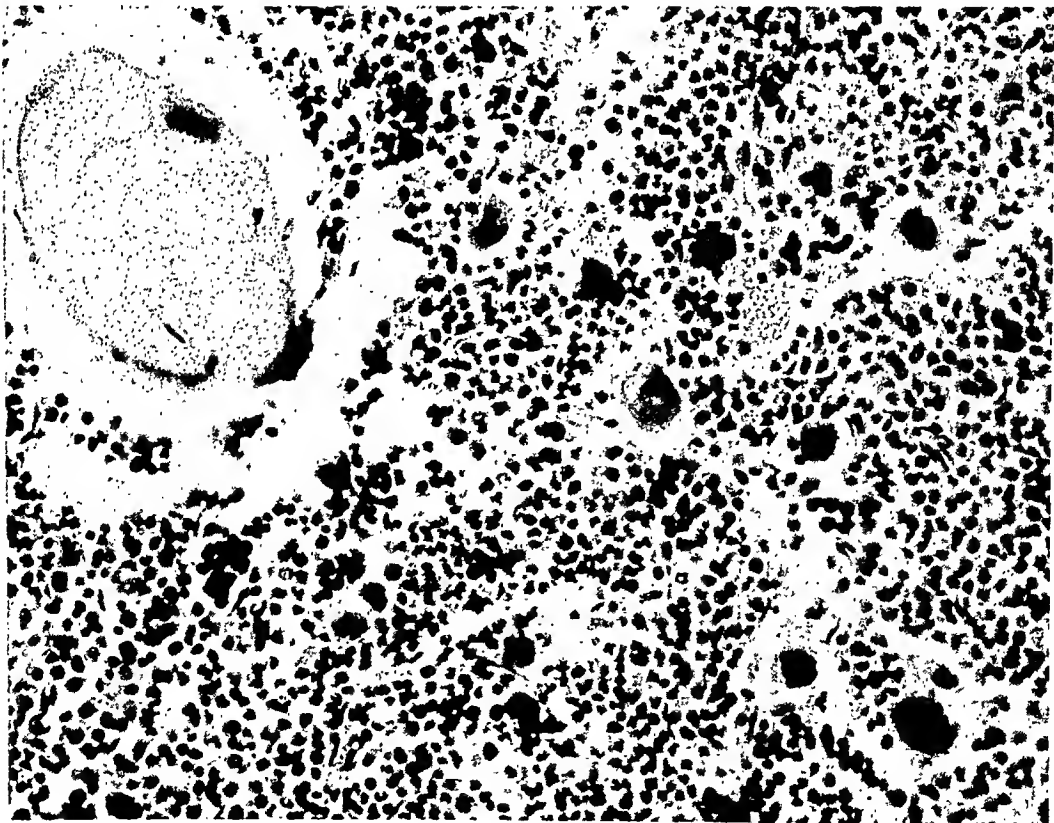
Fig. 8. Case 2. Liver. Intrasinusoidal myelosis with numerous megakaryocytes. × 120.

Fig. 9. Case 2. Liver. Development of megakaryocytes from sinus endothelium. The multinucleated cell in the right half of the field represents an early stage of development. × 650.
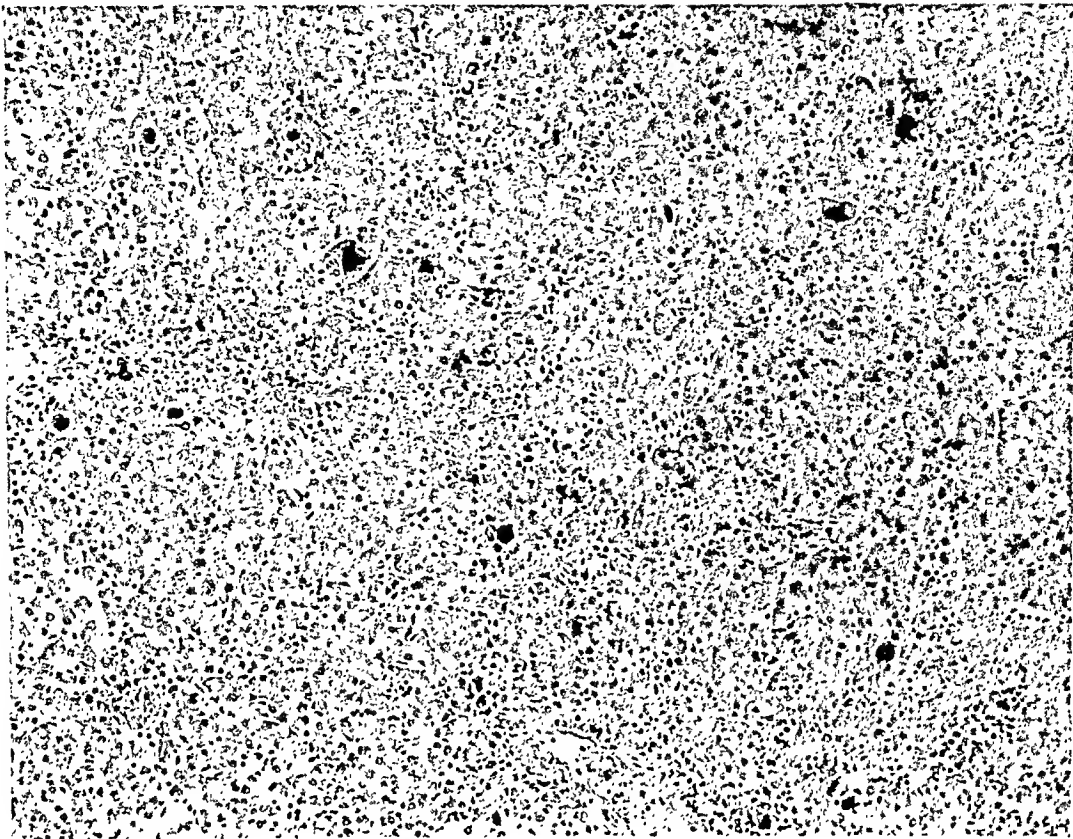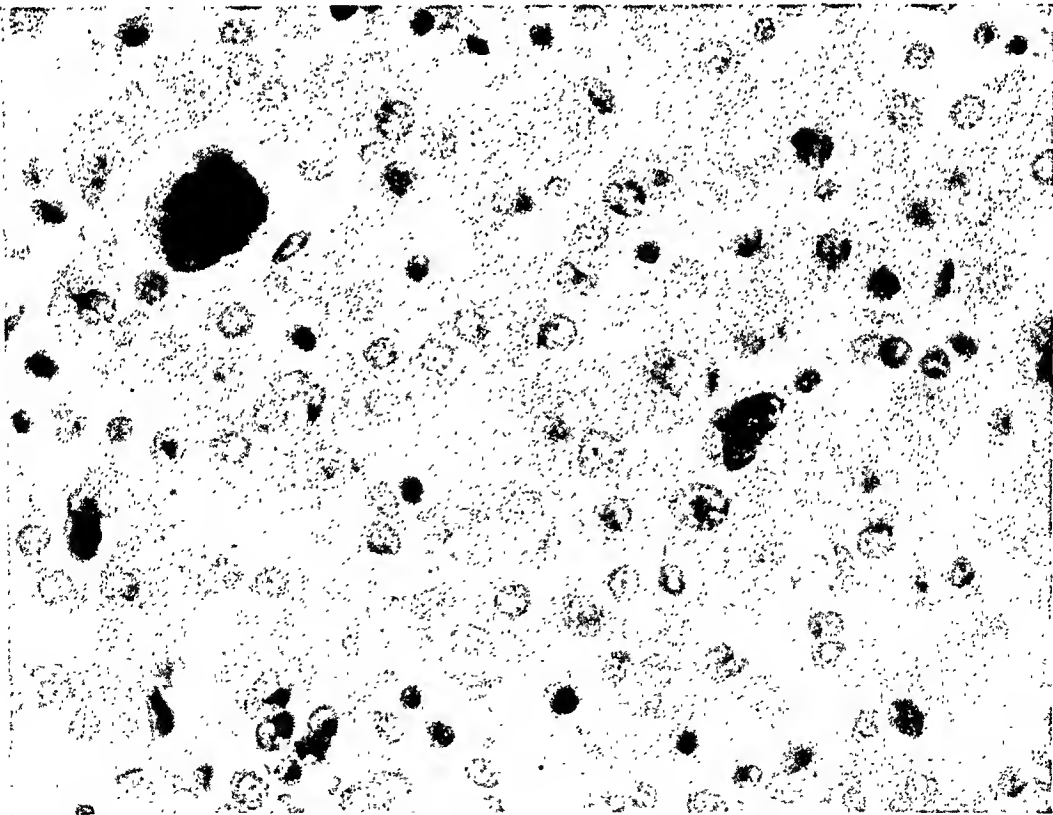
8

9

Heller, Lewisohn, and Palin

Aleukemic Myelosis

Heller, Lewisohn, and Palin

Aleukemic Myelosis
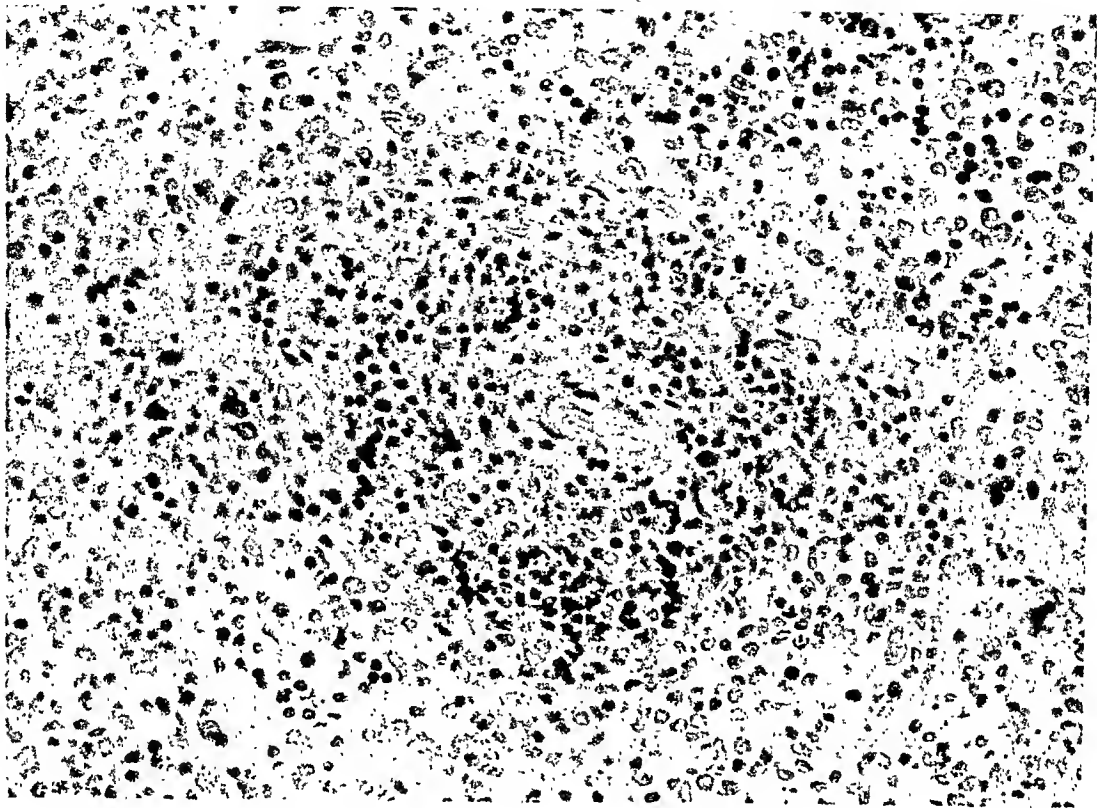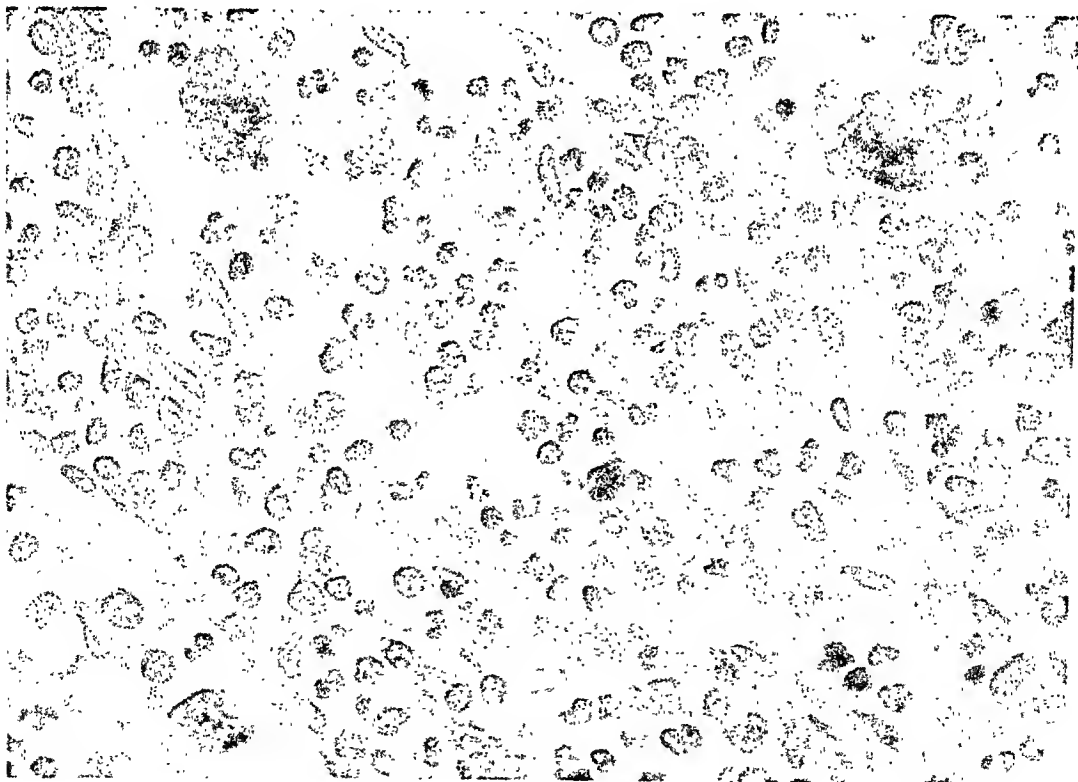
PLATE 55

FIG. 12. Case 3. Bone marrow. Diffuse myeloid hyperplasia. $\times$ 120.

FIG. 13. Case 3. Liver. In the left half of the field the portal stroma is infiltrated with myeloid cells. There is a similar involvement of the sinusoids. $\times$ 300.

Aleukemic Myelosis
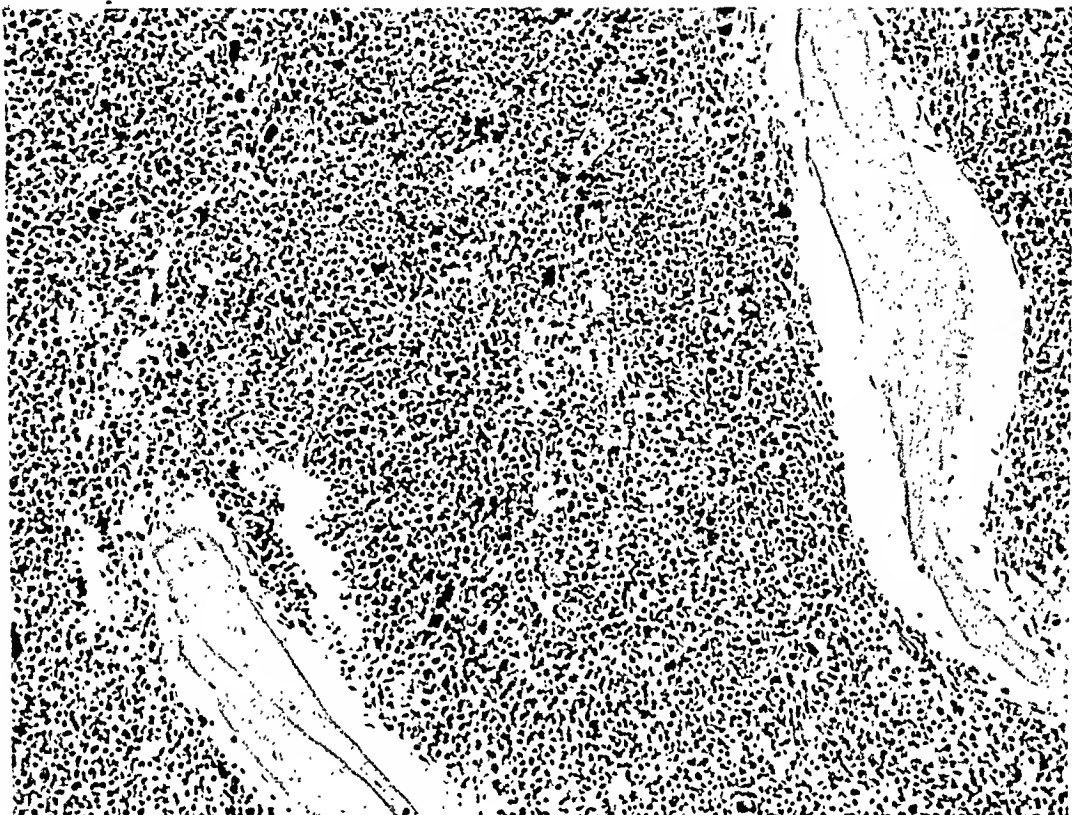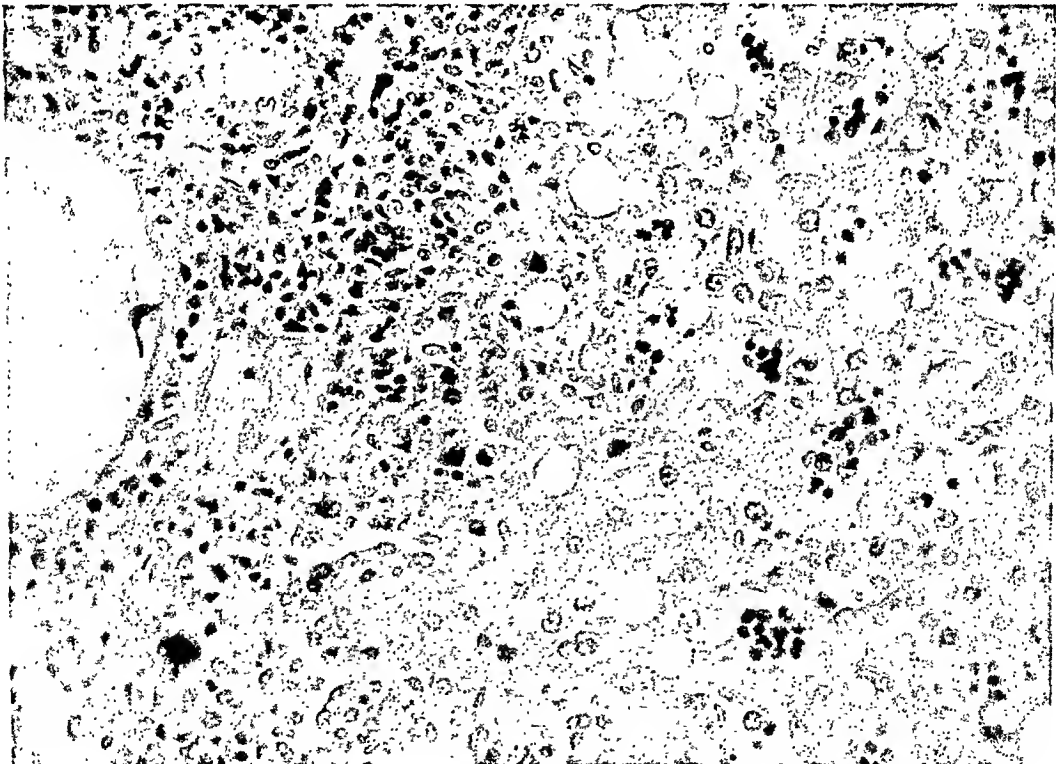
LESIONS FOLLOWING THE USE OF ERTRON IN RHEUMATOID ARTHRITIS [*]

Margaret Bevans, M.D., and Henry K. Taylor, M.D.

(From the Departments of Pathology and Radiology and the Second Medical Division, Goldwater Memorial Hospital, Welfare Island, N.Y.)

The literature is replete with reports of the administration of various preparations of vitamin D, principally in the therapy of rickets and rheumatoid arthritis. Toxic symptoms in the form of anorexia, nausea, headache, polydypsia, and polyuria are known to occur with varying doses. That anatomic lesions must occur is manifest by the urinary changes and by tests showing impaired renal function and chemical alterations in the blood.[1-5] Only five patients whose deaths have been ascribed to large doses of vitamin D have been autopsied and all were infants.[6-9] Because lesions observed at necropsy following the prolonged use of Ertron [†] have not been described previously, the following case report is of interest.

Report of Case

E. P., a white female artist, 63 years old, was admitted to Goldwater Memorial Hospital for the first time in December, 1942. She stated that she had had pains and progressive deformity of the joints of the extremities for 18 years. Until 2½ years before admission she had received no medical treatment. At that time she was given approximately 100 intramuscular injections of gold extending over a period of 1 year. Her condition improved, but had relapsed after 4 months. Otherwise her past history included nothing relevant to her present condition. Physical examination revealed marked deformity and limitation of motion of the joints of the extremities. There was scoliosis of the lower spine with convexity to the left. The heart was not enlarged and the rhythm was regular. The blood pressure was 168/98 mm. Hg.

The patient remained in the hospital with a normal or slightly subnormal temperature for approximately 3 months, during which time she received x-ray therapy to the involved joints in unknown amount. She was discharged as slightly improved.

Her second and final admission occurred in August, 1945. Approximately 18 months prior to readmission she had begun to take three or four capsules (150,000 to 200,000 international units) of Ertron daily and had continued this dosage for 1 year without medical supervision. Six months before her second admission a lesion described as an "infection" developed on the right foot and discharged thick yellowish pus. Following this, numerous abscesses appeared over the dorsa of both feet, the left knee, and the dorsum of the right hand. Generalized pruritus was present but no rash was noted. The patient developed a chronic cough productive of brown but not bloody sputum. The weight loss was severe but indefinite in amount. No information was available concerning the patient's diet.

Physical examination on admission showed an emaciated, elderly female with

marked deformities of the joints. Several small, discrete, nontender lymph nodes were palpable in the axillae, and in the inguinal and femoral regions. There was a draining sinus posterior to the medial malleolus of the right foot and multiple, tender, cystic swellings on the dorsum of the right hand, the left knee, and the left foot. The heart was regular and no murmurs were heard. The blood pressure on admission was 125/85 mm. Hg.

Course. During the patient's stay in the hospital, one of the abscesses was incised and a portion of the wall was examined microscopically. The report was "chronic nonspecific inflammatory reaction with calcification." Culture of the material from the draining lesions yielded *Staphylococcus aureus* on two occasions. This was thought to be a contaminant, but following penicillin therapy the cultures became sterile. The temperature on three occasions rose to 100°F. but averaged

TABLE I
Laboratory Data

	1943	1945
Hemoglobin	12.8 gm.	6 gm.
Red blood cells	4,600,000	2,500,000
White blood cells	7,500	7,800
Erythrocyte sedimentation rates	72 mm. per hr., Westergren	
Fasting blood sugar	107 mg. %	100 mg. %
Blood urea nitrogen	18 mg. %	58 to 107 mg. %
Blood nonprotein nitrogen		172 mg. %
Total protein		6.6 gm. %
Serum albumin		4.9 gm. %
Serum globulin		1.6 gm. %
Calcium		13 to 14.7 mg. %
Phosphorus		6 mg. %
Alkaline phosphatase		9.6 Bodansky units
CO_2-combining power		68 vol. % falling to 35 vol. %
Urine	Alkaline; albumin, trace; glucose, negative; many white blood cells	10 days before death: alkaline; albumin, +; glucose, +; few epithelial cells; few white blood cells (later examinations not recorded)

about 99.4°F. The patient's urea nitrogen rose progressively from 58 on admission to 107. Blood pressure never rose above 125/85 mm. Hg. She became moribund and expired about 1 month after admission.

Summary of Roentgenologic Findings. The roentgenologic examinations of the chest in 1943 and 1945 revealed no calcific deposits in the pulmonary parenchyma. In 1943, the wrists, hands, and knees showed the characteristic changes of rheumatoid arthritis (Figs. 1, 4, and 5). These changes were extensive, with bone decalcification, destructive changes, contractural deformities of the hands, and ankylosis of the wrists. The left knee showed osseous destruction while the right showed cartilaginous destruction only. The vessels about the joints were not calcified and there were no calcific deposits in the soft tissues.

In 1945, the wrists, hands, and knees showed progressive changes due to arthritis. Despite these changes, the bones were denser and presented a more nearly normal appearance than in 1943. In fact, the entire skeleton was richer in calcium content. The vessels about the joints were calcified, notably the pelvic, femoral, popliteal, and tibial arteries. The deposition of calcium in the soft tissues presented striking appearances. The synovial membrane of the left knee was completely outlined, showing its extent, distribution, and lobulations. Calcium was also deposited within

the joint (Figs. 6 and 7). Considerable calcium was deposited in the soft tissues in and about the right wrist, thenar eminence, fingers of the right hand, and in the interosseous membrane of the forearm. Soft tissue swellings about the wrist and on the dorsal aspect of the hand contained calcium (Figs. 2 and 3). There was less calcium in the left hand, the deposit being limited to the proximal interphalangeal articulation of the ring finger; there was destruction and subluxation of the underlying joint.

Similar changes were observed in the elbows. The destructive changes were pronounced and there was calcium deposited in and about the joints, especially on the left side. Soft tissue lobulations about the joints were outlined by calcium salts. The ankles were free of destructive osseous changes but the metatarsophalangeal articulations were not. Large amounts of calcium were deposited in the soft tissues in and about the ankles and in the joints of the feet as well as in the Achilles tendon. A notable collection was present in and about the metatarsophalangeal articulation of the right big toe. A rather unusual collection was observed in the soft tissues of the left leg extending 13 cm. above the ankle, medial and posterior to the fibula (Figs. 8 and 9).

Comparative study of roentgenographs of the lumbosacral spine in 1943 and 1945 showed no marked changes. There were no calcific deposits in or about the shoulders.

Necropsy

Necropsy was performed 13 hours after death.

The deformities of the joints and other parts of the body were those noted on physical examination. The mouth was edentulous. Discrete but small lymph nodes were present in each axilla. Thick, chalky material was seen at the sternoclavicular joint and at several of the costochondral junctions. Fibrous adhesions were present over both apices and there was a rim of dense parenchyma beneath the apical pleura on both sides. This extended to a lesser degree around the adjacent periphery of the lungs. A few, firm, light pink areas bulged above the surface in all except the right middle lobe. The lungs were rust-colored and exuded large quantities of frothy material. The peribronchial lymph nodes were anthracotic.

The heart with the densely adherent pericardium weighed 360 gm. The surfaces were shaggy. The endocardium of the left auricle was not thickened. The mitral valve was not deformed and the chordae tendineae were delicate. The remaining valves were normal. There was a moderate amount of diffuse sclerosis of the coronary arteries but the lumina were patent. The ostia of the renal arteries were narrowed by sclerotic plaques. The pancreas was irregular, the normal parenchyma being replaced in the head and tail by ill defined, firm, yellow masses which on section exuded from their centers small amounts of thick, putty-like material. No dilatation of the ducts was noted and the remaining parenchyma was apparently normal. The kidneys were small, weighing 60 gm. each. The surfaces were smooth but there were numerous punctate red areas. The cortices were reduced and the pelves, ureters, and urinary bladder were normal.

Cultures of the fluctuant areas of the right hand, left foot, pancreas, and fluid from the knee joint were sterile.

On microscopic examination of the left auricle, the endocardium was found to be slightly thickened by edema and connective tissue. The subendocardial portion of the auricular myocardium was replaced by a series of poorly circumscribed granulomatous lesions (Fig. 10). These were made up of partially calcified, amorphous, necrotic material surrounded by hyaline connective tissue, radially arranged fibrocytes, bizarre-shaped pyknotic nuclei, and a few multinucleated giant cells. In the cytoplasm of these were particles of calcium. The myocardial fibers at the periphery of the nodules were fragmented but there was little cellular reaction about them (Fig. 11). That which was present was composed of a few lymphocytes and Anitschkow myocytes. The lesions were poorly vascularized, but in the myocardium surrounding them were several capillaries. The mitral valve showed a few vessels with intimal thickening, but was otherwise not unusual. The mitral ring contained calcium. The endocardium of the left ventricle was normal. The myocardium showed a few areas of perivascular scarring which replaced small numbers of adjacent myocardial fibers. Most of the epicardial fat of the left ventricle was replaced by a thick fibrous band of dense but well vascularized connective tissue. The surface was covered by fibrinoid exudate which was partly organized. A few polymorphonuclear leukocytes were found in the fibrous tissue.

Numerous sections through various parts of the heart showed nothing unusual. There was moderate perivascular scarring and a small amount of intimal proliferation in the small branches of the coronary arteries, but no Aschoff nodules were seen.

Sections from the peripheral portions of both lungs showed replacement of the normal architecture by fibrous tissue (Fig. 12). In some areas the alveolar septa persisted but were greatly thickened and the alveoli were reduced in size. Within the fibrous tissue were multinucleated giant cells and masses of calcium. Aggregations of necrotic polymorphonuclear leukocytes and fibrin persisted in the midst of the fibrous tissue. The bronchial epithelium in a few places contained calcium. Beneath the epithelium were spicules of calcium which had eroded the epithelium and projected into the lumen. The lumina also contained purulent exudate and coagulum. The alveolar lining, when present, consisted of a single layer of flat cells outlining the irregular dilated spaces. Some of the septa were completely calcified (Fig. 13). The vessel walls were thickened by intimal proliferation of connective tissue and calcium was deposited in a rim about the adventitia. Other

sections from the right apex showed masses of necrotic purulent exudate in hyalinized scar tissue. Fresh exudate with colonies of gram-positive cocci was noted in one area.

The thyroid contained numerous nodules composed of acini which varied in size and were surrounded by connective tissue. The small amount of calcium deposited in the scar tissue was not unusual.

One parathyroid appeared normal except for a minute cyst filled with colloid. A nodule removed as parathyroid was composed of spicules of calcium, foreign body giant cells containing particles of calcium, and fibrocytes (Fig. 14).

In the pancreas there was extensive necrosis, both of the interstitial fat and parenchyma. In abscess cavities were masses of fibrin and polymorphonuclear leukocytes. All stages of healing were seen in various sections. In large areas the parenchyma had been replaced by dense scar tissue. In these areas the ducts were dilated and filled with amorphous pink coagulum but the epithelium was normal (Fig. 16). Giemsa's and Brown's * stains failed to reveal bacteria in the areas of acute exudation. There were no calcium deposits.

Some renal glomeruli were small and shrunken, and a few were hyalinized with the capsules of Bowman thickened. The afferent arterioles and basement membranes were also slightly thickened by hyalinized connective tissue in a few areas. There was reduction of both cortex and medulla due to atrophy and disappearance of tubules, but the renal papillae were not blunted. Scattered throughout the parenchyma, but principally in the medulla, were minute foci of necrotic polymorphonuclear leukocytes, lymphocytes, and plasma cells which seemed to represent necrotic tubules filled with polymorphonuclear leukocytes, the walls of which had ruptured. In many places these small abscesses were healing. All tubules were disarranged; many were distended with various types of casts, others showed necrotic walls, and still others, hyperplasia of the epithelium. Many tubules were atrophic and filled with hyalinized and granular coagulum. A few showed calcified casts (Fig. 15). The interstitial tissue was edematous but remarkably free of infiltration except about the granulomatous lesions. The walls and pelvic epithelium were not unusual. The renal arteries showed moderate sclerosis, but calcium deposits were lacking. With von Kossa's stain large amounts of calcium which were not apparent in the hematoxylin and eosin preparations were demon-

---

* Brown, J. H., and Brenn, L. A method for the differential staining of gram-positive and gram-negative bacteria in tissue sections. *Bull. Johns Hopkins Hosp.*, 1931, 48, 69–73.

strated in the granular casts and in the epithelium and basement membranes of the tubules. The calcium occurred in both the convoluted and collecting tubules.

The lymph nodes revealed focal areas of necrosis similar to those found in the pancreas and kidneys. The sinusoids were filled with polymorphonuclear leukocytes.

Sections of the chalky deposits about the ribs and clavicles showed masses of amorphous material containing calcium which spread into the surrounding muscle. Many of the individual muscle fibers showed partial calcification. No osteoporosis or evidence of acute inflammation was noted.

Sections of the aorta and its major branches showed sclerotic changes of no more marked degree than might be expected in any patient of the same age.

With Brown's and Gram's stains no bacteria were found in the lymph nodes, heart, and kidneys.

Anatomical diagnoses included: Chronic rheumatoid arthritis; calcinosis involving the periarticular tissues of the extremities, the sternoclavicular joints, the costosternal articulations, the subcutaneous tissue, the myocardium of the left auricle, the lungs, and the kidneys; lobular pneumonia; acute and chronic pancreatitis with abscess formation.

COMMENT

The cardiac lesions observed in the left auricle are not essentially different from those described by Baggenstoss and Rosenberg[10] as occurring in rheumatoid arthritis, except that the calcification in this instance is much greater and the giant cells contain particles of calcium. Involvement of the myocardium and not of the adjacent auricular endocardium is unusual for a lesion of rheumatic origin, yet the situation in the left auricle suggests this causal factor. It is probable that this is a lesion similar to those found in the other organs. It does not resemble the degeneration and vacuolization of the myocardial fibers mentioned by Malmberg[6] as occurring following cod-liver oil therapy. The pericardial lesions had nothing to distinguish them from an organizing fibrinous pericarditis of long duration. No mention is made of the pericardium in any other cases. In this instance the pericarditis may be a manifestation of long-standing uremia, although its severity and wide distribution make this unlikely.

Despite evidence in the roentgenograms of increase in calcium in the peripheral vessels of the lower extremities, sections of the aorta and of the renal, iliac, and hypogastric arteries showed only arteriosclerosis of mild degree.

The lesions in the lung were apparently unique. It may be argued that the necrotic foci surrounded by polymorphonuclear leukocytes in the midst of the fibrous tissue are an indication of organizing pneumonia which in turn has caused the fibrous tissue proliferation. Be that as it may, we have never observed this bizarre type of calcification in other pneumonias. The location of the calcium suggests that its deposition was influenced by the rapid change in pH which occurs in the alveolar spaces.

The presence of calcium in the skin and in the periarticular tissues in conjunction with a hypercalcemia coincides with the observations of many authors.[2,3,5]

The density of all the bones was increased following the use of Ertron. This suggests that the excessive calcium was being derived from sources other than bone.

Calcium deposits have been noted grossly in the kidneys of both children and experimental animals which have received large amounts of vitamin D. Localization in and about the tubule has been recorded by many observers.[6-9,11] Calcium deposition in the kidney was not as conspicuous in the case reported here. It was demonstrated in the lumina as well as the walls of the tubules. The glomerular changes were slight. The pelves and ureters showed no evidence of previous or recent damage. The localization of the acute lesions to the tubules is interesting to note, for if this were a disseminated hematogenous lesion of a septic nature, one would expect the glomeruli to be involved. We are aware that the kidneys may have been damaged by the therapeutic use of gold, but the fact that the urea nitrogen of the blood was normal 18 months after the cessation of such therapy suggests that the damage was not severe. The lesions of the tubules were of sufficient severity to account for the mounting urea nitrogen of the blood as observed clinically.

Since impaired renal function and nitrogen retention are known to occur in connection with the toxic action of vitamin D, both in lower animals and man, and since these signs developed in this patient following the prolonged use of this substance, it seems fair to assume that vitamin D played some rôle in the clinical picture of renal insufficiency.

The pancreatic lesions were similar to those seen in the kidney, except that calcification was lacking. The persistent finding of 1 plus glucose in urinalyses may have been due to a direct effect upon the islets of Langerhans, although in the sections examined these seemed normal and numerous. No mention is made of pancreatic lesions in necropsy reports of children, or in animal experiments.[12]

The nodule removed as parathyroid was difficult to interpret. It

showed only fibrous tissue, calcium, and foreign body giant cells resembling those seen in the lungs and skin. The thyroid gland showed no such reaction, so that contiguity with this structure seemed unlikely. The other parathyroid was normal.

It is possible that this patient had a low-grade sepsis with multiple granulomatous lesions occurring in various parts of the body, the causal agent of which was obscure. However, hypercalcemia, and deposition of calcium in the periarticular tissues, alveolar septa, left auricle and kidney tubules can scarcely be attributed to sepsis. We are tempted to include the lesions of the pancreas and lymph node, and the uncalcified pulmonary lesions as additional manifestations of the same disturbance.

SUMMARY AND CONCLUSIONS

Unsupervised use of Ertron in a patient suffering from rheumatoid arthritis led to hypercalcemia, and calcium deposition in the periarticular and subcutaneous tissues, lungs, heart, and kidneys.

There were chronic and acute granulomatous lesions in the lungs, the pancreas, the kidneys, and the lymph nodes, the nature of which was obscure. It is suggested that these lesions also are related to the use of Ertron.

Extensive damage to the renal tubules formed an anatomic basis for clinical renal insufficiency.

Since this paper was submitted for publication two reports have appeared describing similar lesions observed at necropsy in adults,. following extensive use of vitamin D preparations. (Bauer, J. M., and Freyberg, R. H. Vitamin D intoxication with metastatic calcification. *J. A. M. A.*, 1946, *130*, 1208–1215. Mulligan, R. M. Metastatic calcification associated with hypervitaminosis D and haliphagia. *Am. J. Path.*, 1946, *22*, 1293–1305.) The opinions expressed by these authors corroborate the statement that various acute and chronic lesions as well as calcification are related to excessive vitamin D ingestion.

REFERENCES

1. Freyberg, R. H. Treatment of arthritis with vitamin and endocrine preparations; emphasis on their limited value. *J. A. M. A.*, 1942, *119*, 1165–1171.
2. Tumulty, P. A., and Howard, J. E. Irradiated ergosterol poisoning; report of two cases. *J. A. M. A.*, 1942, *119*, 233–236.
3. Danowski, T. S., Winkler, A. W., and Peters, J. P. Tissue calcification and renal failure produced by massive dose vitamin D therapy of arthritis. *Ann. Int. Med.*, 1945, *23*, 22–29.
4. Hench, P. S., Bauer, W., Ghrist, D., Hall, F., Holbrook, W. P., Key, J. A., and Slocumb, C. H. The present status of rheumatism and arthritis: review of American and English literature for 1936. *Ann. Int. Med.* 1938, *11*, 1089–1247.
5. Freeman, S., Rhoads, P. S., and Yeager, L. B. Toxic manifestations associated with prolonged Ertron ingestion. *J. A. M. A.*, 1946, *130*, 197–202.

6. Malmberg, N. Some histological organic changes after cod-liver oil medication. *Acta paediat.*, 1928, **8**, 364–374.

7. Putschar, W. Über Vigantolschädigung der Niere bei einem Kinde. *Ztschr. f. Kinderh.*, 1929–30, **48**, 269–281.

8. Thatcher, L. Hypervitaminosis-D, with report of a fatal case in a child. *Edinburgh M. J.*, 1931, **38**, 457–467.

9. Thatcher, L. Hypervitaminosis D. *Lancet*, 1936, **1**, 20–22.

10. Baggenstoss, A. H., and Rosenberg, E. F. Unusual cardiac lesions associated with chronic multiple rheumatoid arthritis. *Arch. Path.*, 1944, **37**, 54–60.

11. Chown, B., Lee, M., Teal, J., and Currie, R. On the experimental production of nephritis in rats by means of parathyroid hormone and of vitamin D. *J. Path. & Bact.*, 1939, **49**, 273–290.

12. Reed, C. I., Struck, H. C., and Steck, I. E. Vitamin D. University of Chicago Press, Chicago, 1939, pp. 151–210.

[*Illustrations follow*]

DESCRIPTION OF PLATES

PLATE 56

FIG. 1. Right wrist, 1943. Advanced rheumatoid arthritic changes. Fusion of the residuals of the carpal bones with ankylosis. Contraction deformities of fingers; destructive changes involving the metacarpophalangeal articulations of the right hand. Decalcification of bones.

FIGS. 2 and 3. Right wrist and hand, 1945. Extensive progression of the lesion. Of note is the irregular deposition of calcium in and about the bones of the right wrist and hand, in the periarticular soft tissues, the thenar eminence, the soft tissues of the fingers, and in the interosseous space. Density of the bones is greater than in Figure 1.

PLATE 57

FIGS. 4 and 5. Left knee, 1943. Destruction of articulating cartilages and subchondral bone with bone sclerosis on contiguous surfaces of femoral and tibial condyles. Effusion in the joint and suprapatellar bursa. Moderate decalcification. No calcific deposits in the soft tissues. The vessels are not calcified.

FIGS. 6 and 7. Left knee, 1945. Of note are the progressive arthritic changes and increased bone density. Lateral subluxation and dense lobulated calcific deposit in the soft tissues corresponding to the synovial membrane of the knee joint. Calcific deposits in the soft tissues and in the walls of the femoral, popliteal, and tibial arteries. Increased calcification in the upper end of the tibia.

PLATE 57

4

5

6
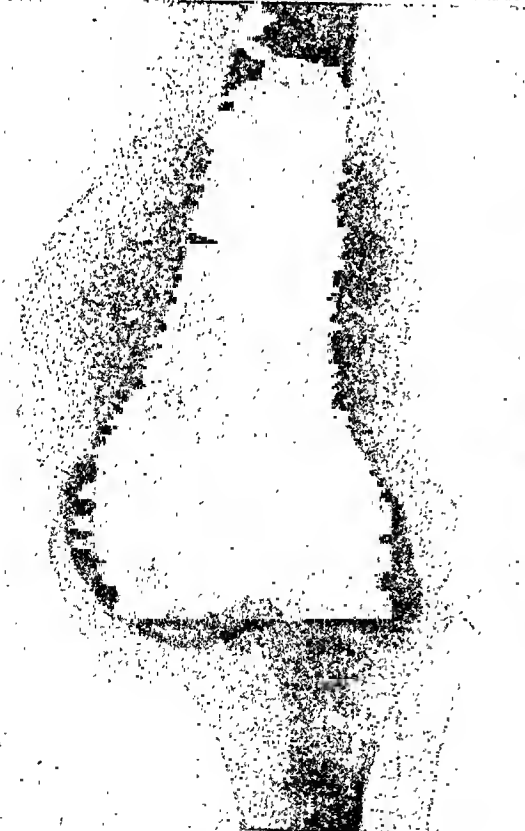
7
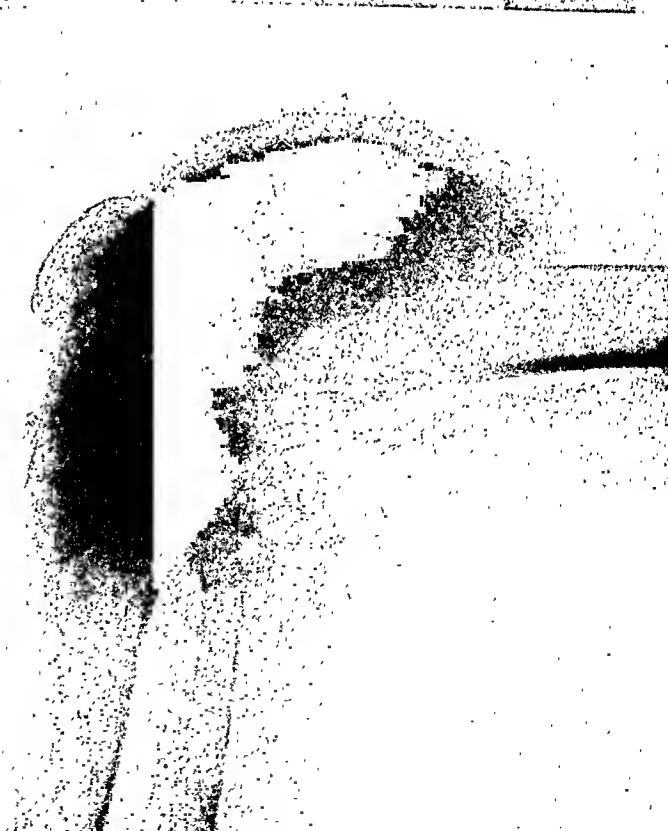
Bevans and Taylor

Effects of Ertron

PLATE 58

FIGS. 8 and 9. Left ankle and foot, 1945. Calcium deposited in the soft tissues in the lower third of the leg, about the tarsal bones and ankle. Large lobulated collections of calcium in the soft tissues posterior and medial to the shaft of the fibula.

FIG. 10. Left auricle showing extensive calcification of subendocardial myocardium. Von Kossa's stain. ✕ 40.

Bevans and Taylor

Effects of Ertron
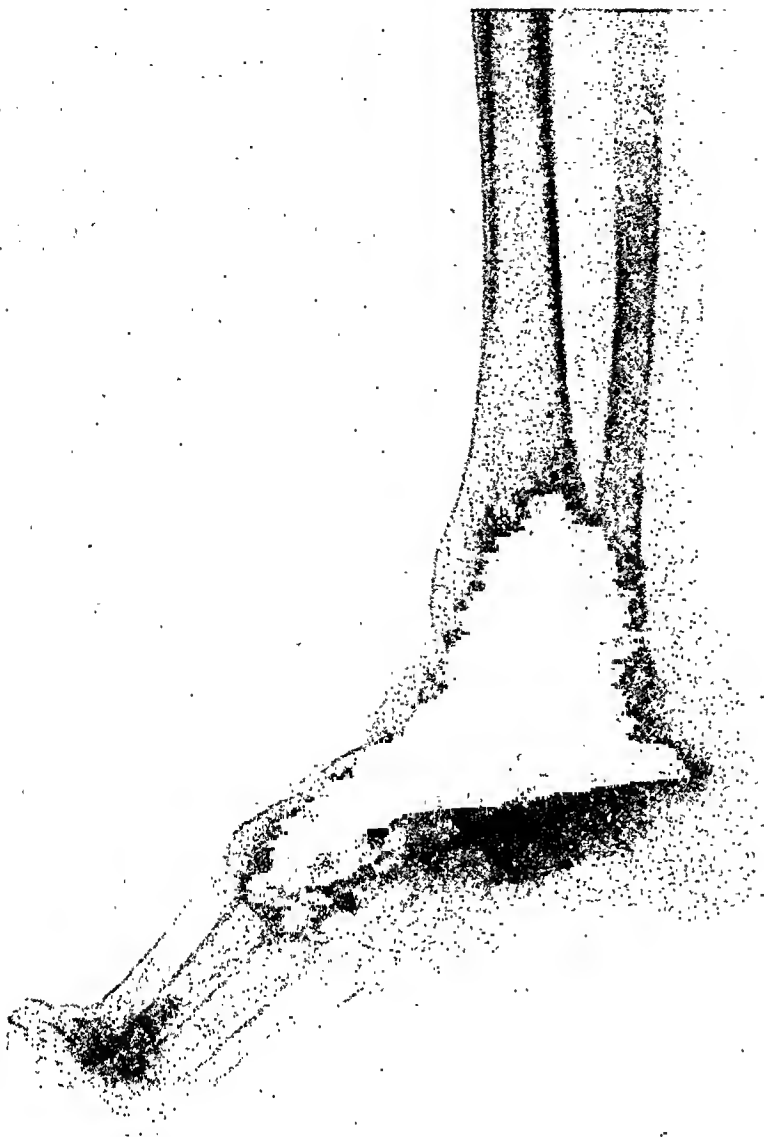
PLATE 59

Fig. 11. Higher magnification of granulomatous lesions replacing the myocardium of the auricle. Hematoxylin and eosin stain. $\times$ 165.

Fig. 12. Section of lung showing extensive fibrosis. Hematoxylin and eosin stain. $\times$ 35.

Bevans and Taylor

Effects of Ertron
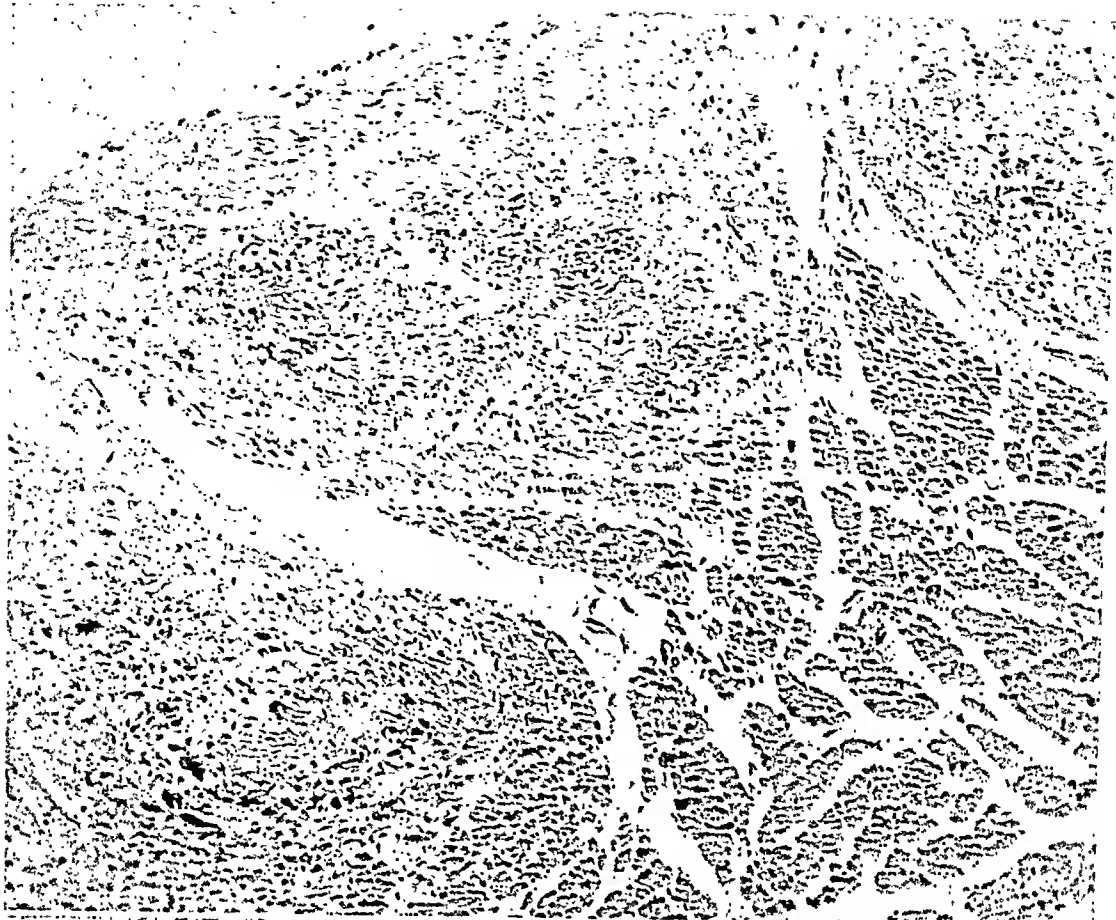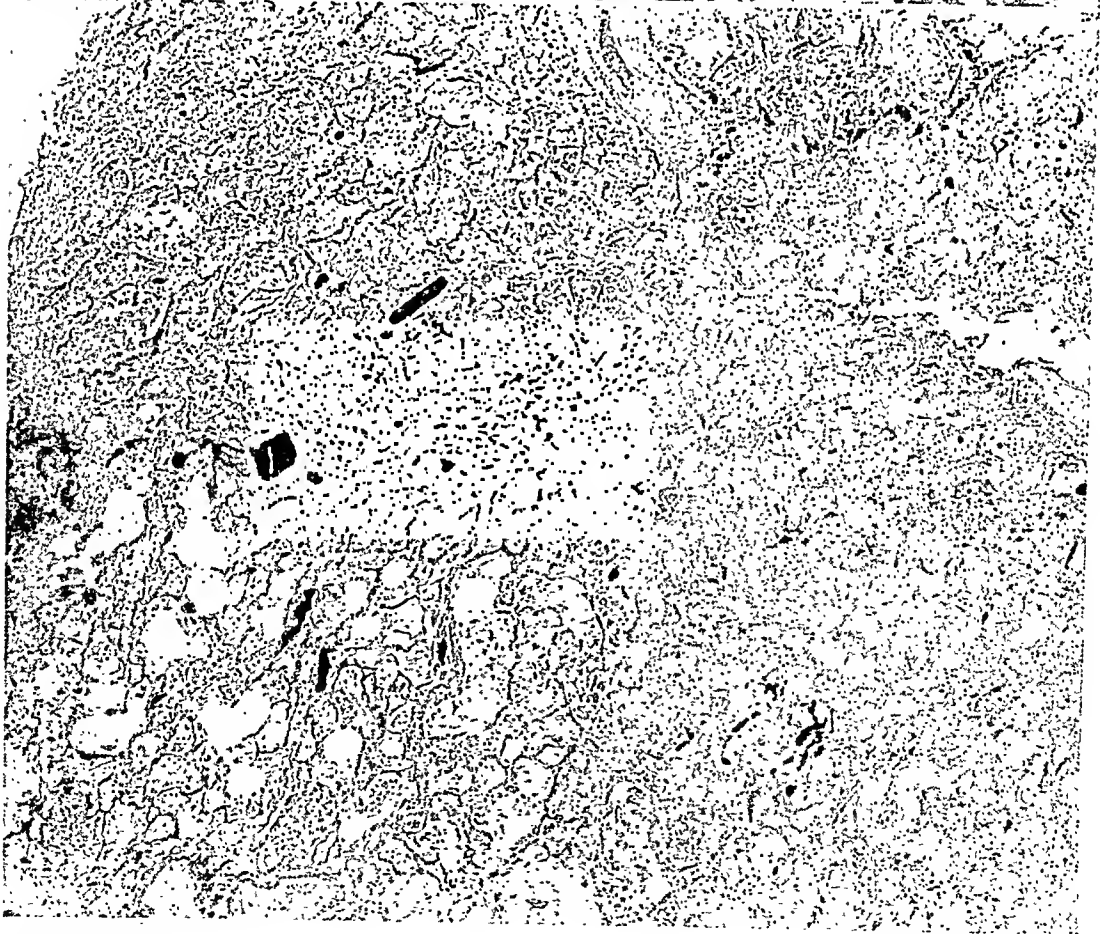
FIG. 13. Higher magnification of lung. Two spicules of calcium in the alveolar septum appear to the left of the center. A foreign body giant cell is present at the lower right. Hematoxylin and eosin stain. $\times$ 515.

FIG. 14. Tissue removed as parathyroid. Granulomatous lesion typical of that seen also in the heart and lungs. Of note are the foreign body giant cells with particles of calcium within the cytoplasm and free calcium throughout the lesion. Hematoxylin and eosin stain. $\times$ 615.

13

14

Bevans and Taylor

Effects of Ertron
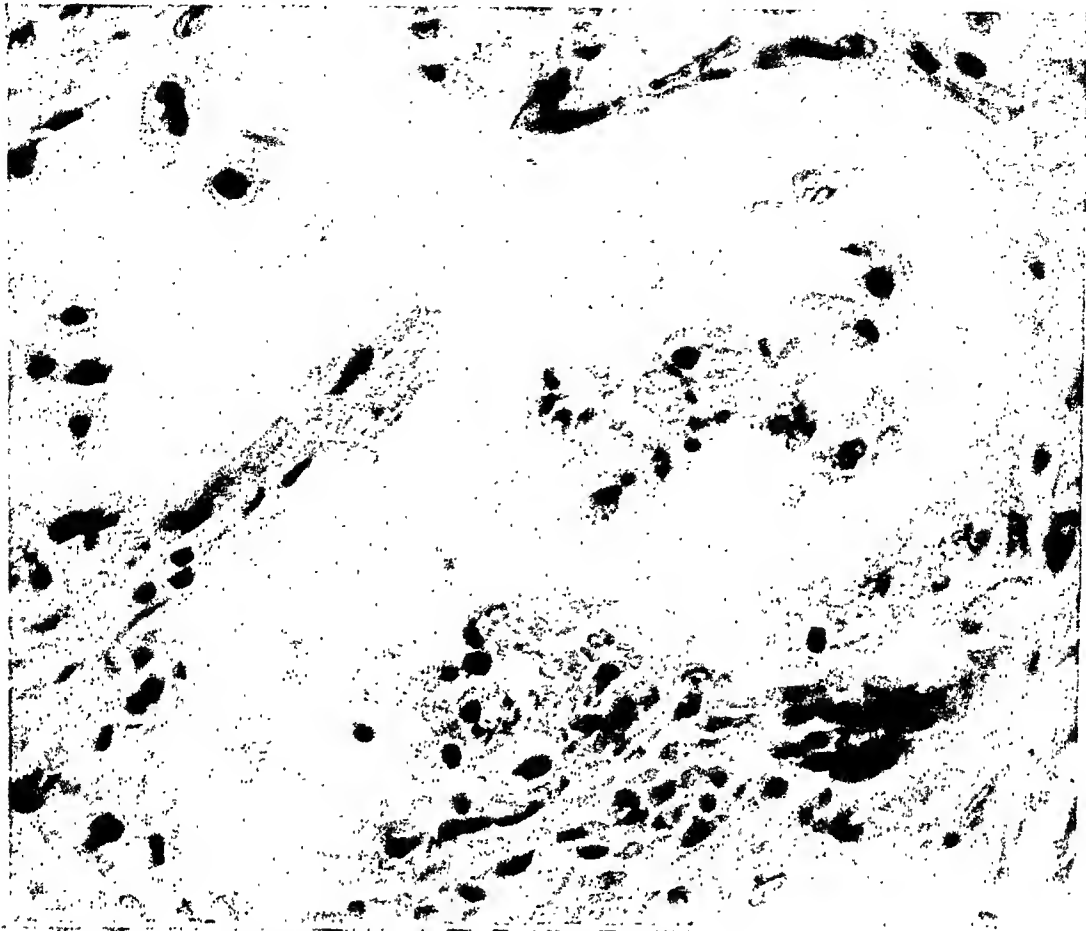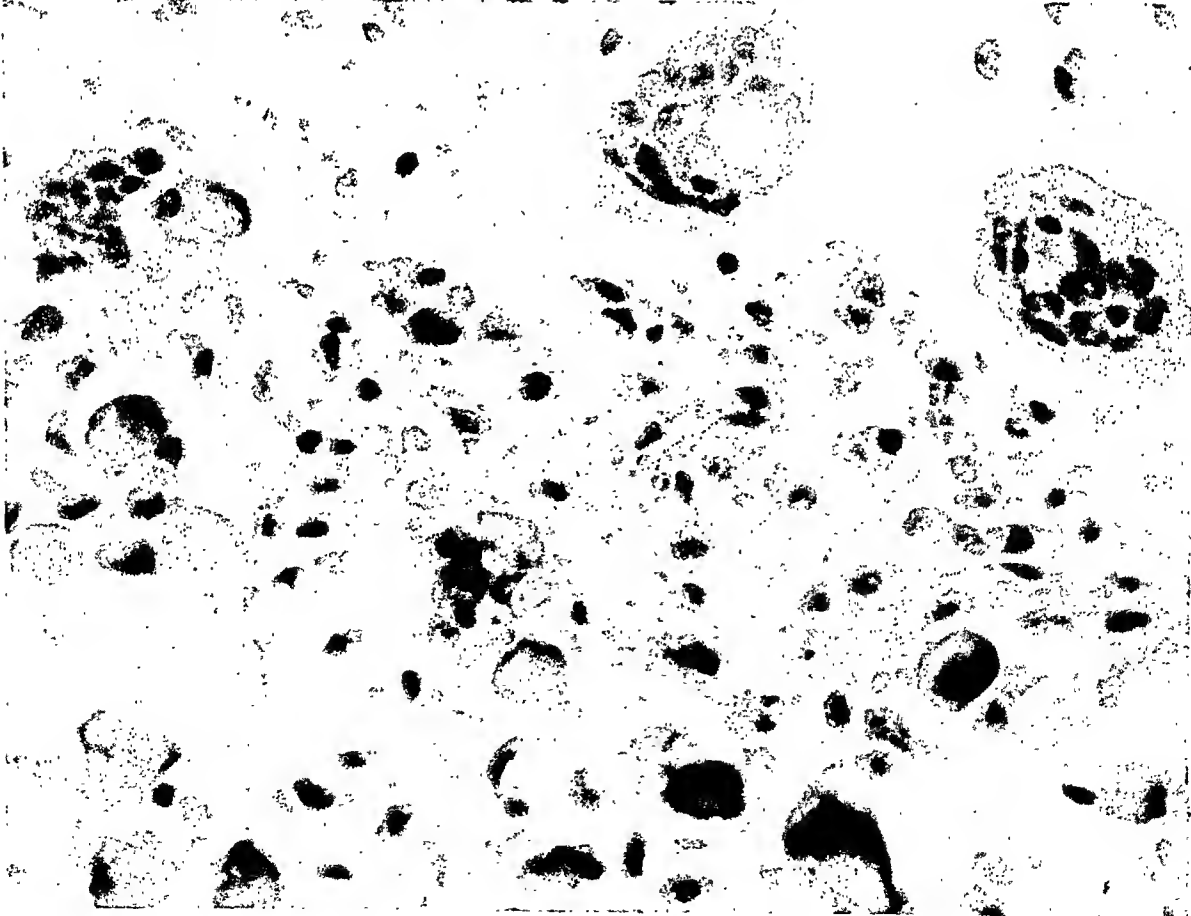
PLATE 61

FIG. 15. Kidney showing atrophic tubules and calcified cast at the right. Hematoxylin and eosin stain. $\times$ 125.

FIG. 16. Pancreas showing necrosis and fibrosis. Hematoxylin and eosin stain. $\times$ 40.
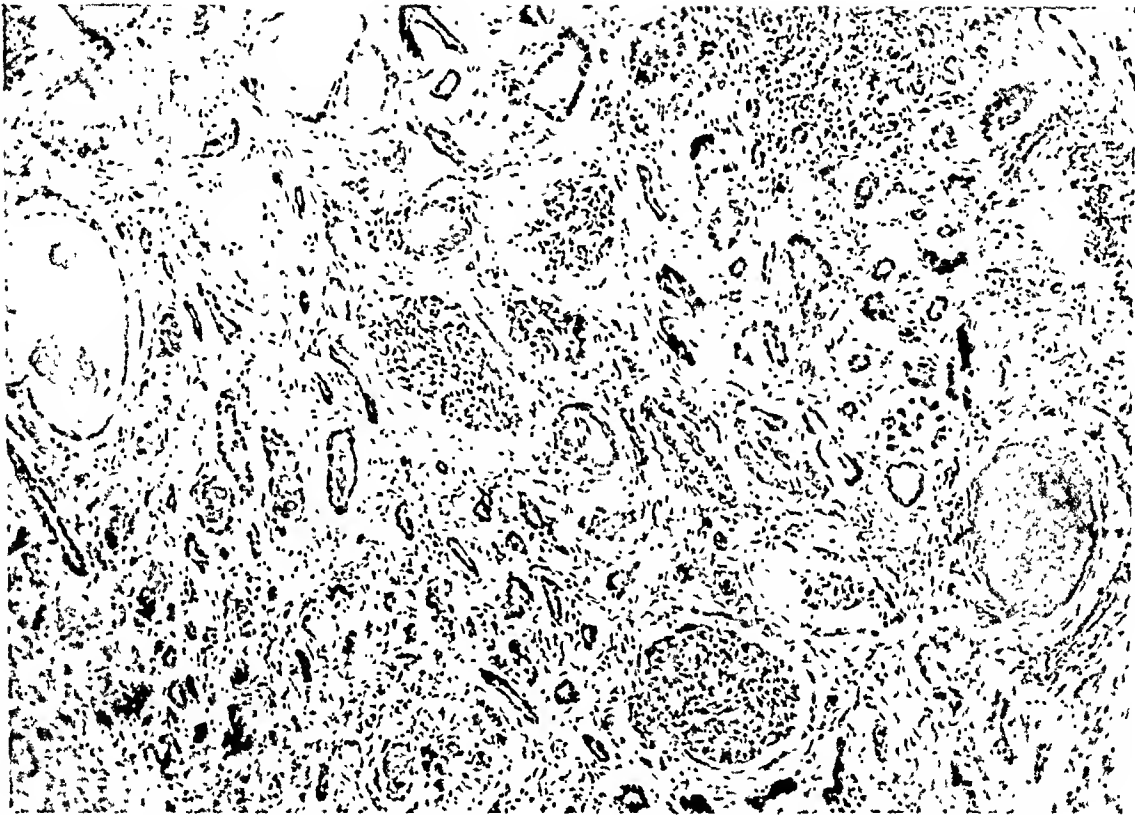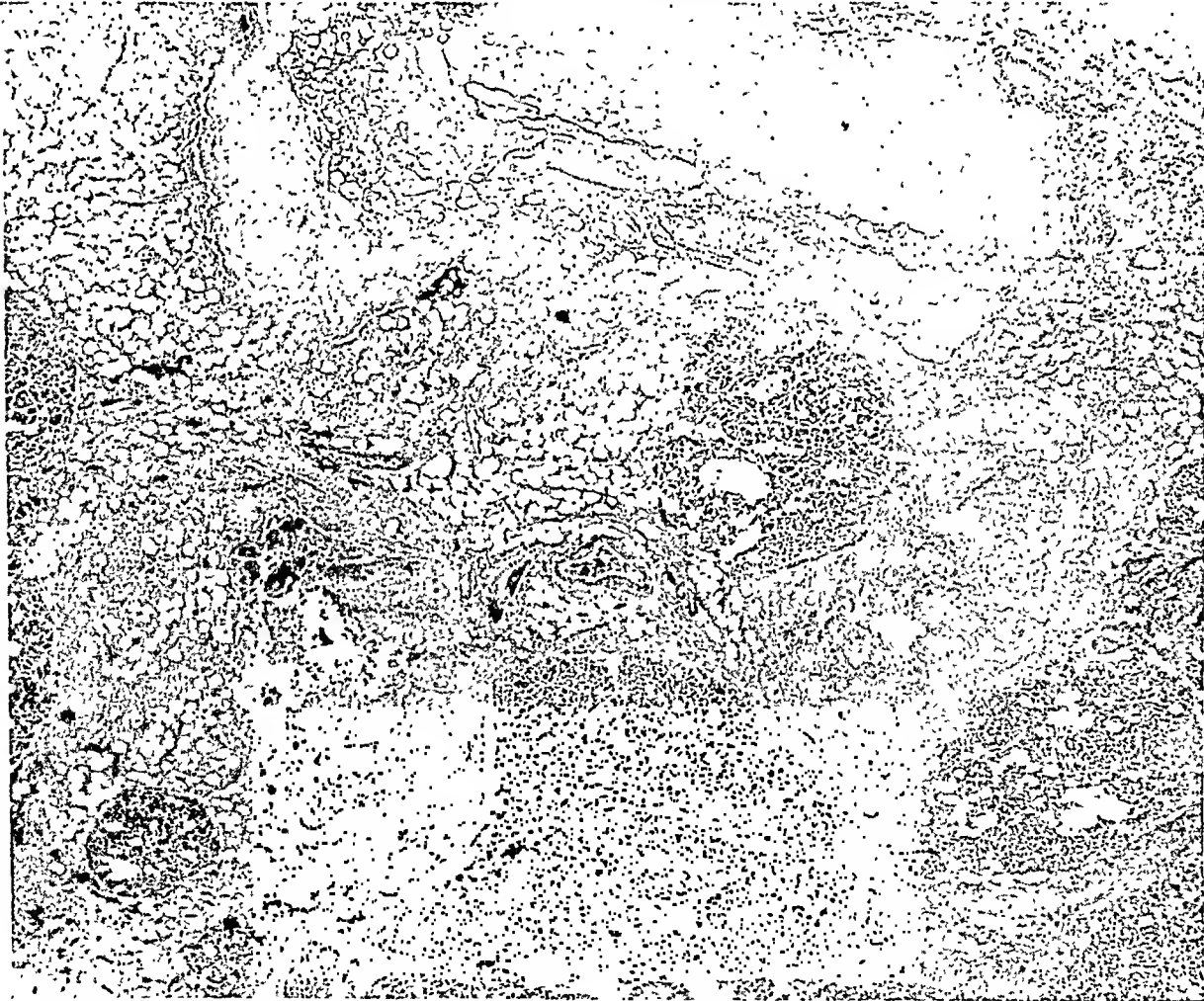
RADIO-AUTOGRAPHIC STUDIES OF THE DISTRIBUTION OF LEWISITE AND MUSTARD GAS IN SKIN AND EYE TISSUES *

Dorothy J. Axelrod, M.A., and Joseph G. Hamilton, M.D.

(*From the Crocker Radiation Laboratory, University of California, Berkeley, and the Division of Medicine and Radiology, University of California Medical School, San Francisco, Calif.*)

Radio-autography is a technic which utilizes the photographic property of radio-activity and is useful in studying the histologic distribution of an element or compound in the tissues of living organisms. Following the administration of a radio-active element or compound to an organism, samples of tissues are removed, and histologic sections are prepared. The sections of tissue, mounted on microscopic slides, are placed in contact with x-ray film. After a suitable period of exposure the film is removed, developed, and the sections are stained. The regions of darkening on the film represent areas where accumulation of the radio-active material has occurred in the tissue section. A correlation of the microscopic anatomy of the tissue and the distribution of the accumulated radio-active element or compound may be established.

The basic principles involved are as follows: (1) A total of from 2 to 10 million beta particles from the section must strike each square centimeter of the film to produce an adequate blackening. (2) The radio-active element or compound must be firmly fixed in the tissue so that the various conditions to which the tissue is subjected during the preparation of the sections will not leach it out. (3) The sections should be less than 10 μ in thickness to obtain maximum resolution. Satisfactory results are obtained with sections ranging in thickness from 5 to 8 μ; sections which are thinner do not give significantly superior resolution. The resolution obtainable varies to a considerable degree but under the very best conditions is 25 μ, which precludes the possibility of the study of the internal structure of individual cells. (4) The half-life of the radio-active agent used must be sufficiently long to permit the preparation of the sections, and there must remain an adequate radio-activity to produce a satisfactory radio-autograph.

The distribution in skin and eye tissues of two war gases, mustard and lewisite, has been studied using this technic. These substances were labeled with radio-active sulfur and radio-active arsenic, respectively.

METHODS

Skin and eye tissues were exposed to the two gases. The skin was thoroughly washed with petroleum ether to remove the residual applied material. The tissues were removed from the experimental animals after varying intervals and fixed in formalin. Skin was embedded in paraffin. Eye sections were prepared both by the paraffin technic and by freezing, the better sections being obtained by the latter method. Paraffin sections were cut 10 μ in thickness, while the frozen sections were approximately 15 μ. The sections were mounted on microscopic slides, and the paraffin was removed by xylol. The slides were then dipped in a dilute solution of celloidin and set on edge to dry. This procedure served to remove the paraffin from the sections since it would otherwise smear the emulsion, and to cover the slides with a thin, protective layer. Some experiments were done using unfixed, frozen tissues. Such tissues were frozen with dry ice immediately after excision and then cut on a freezing microtome.

Each slide was covered with a piece of no-screen x-ray film (Agfa), then carefully wrapped in black paper to exclude light, and finally placed under a lead weight in order to hold the film in close contact with the sections. After a suitable exposure (in these experiments, the times varied from 1 to 3 weeks for optimum blackening), the films were developed, the sections washed in ether-alcohol to remove the celloidin, and stained with hematoxylin and eosin. Each stained slide and its corresponding radio-autograph was examined microscopically; the regions of darkening on the radio-autograph were correlated with the histologic structures in the corresponding area on the section. The areas of darkening on the film correspond to the regions in the sections where the lewisite or mustard gas was accumulated.

It must be emphasized that radio-autographs indicate only the presence of the radio-element; this is probably also an index of the distribution of the compound, but the possibility must be considered that the radio-element could become separated from the original molecule by interaction with animal tissue fluids.

RESULTS

Studies with Mustard Gas on Human Skin

Two experiments were run with mustard gas labeled with radioactive sulfur, S^{35}, containing 5 microcuries of S^{35} per mg. of mustard and applied on human skin. The first exposure was for 10 minutes to 475 μg., and the second was for 15 minutes to 475 μg. of mustard; in each case, the area exposed was 0.43 square cm., and biopsy specimens of these areas were taken 24 hours after exposure. Essentially similar

results were obtained in both experiments. Radio-actively tagged mustard was fixed in the epidermis and in the corium (Fig. 1). The epidermal concentration was slightly higher than that in the corium. Hair follicles were seen rarely and it was difficult to determine whether or not they fixed mustard. In the dermis, the blackening of the autographs was so great as to make it difficult to determine the specific concentration by blood vessels. There does not appear to be any correlation between the radio-activity present and cell injury due to mustard since a large amount of fixed sulfur radio-activity was present despite the fact that few cells were injured. This effect may be due to the fact that morphologic evidence of injury requires longer than 24 hours for development after exposure to the gas.

Studies with Lewisite on Human Skin

Two experiments on human skin were run with lewisite labeled with radio-arsenic, As[74], and containing about 10 microcuries of As[74] per mg. of lewisite; the first exposure was for 10 minutes to 475 μg. of lewisite and the second for 15 minutes to 475 μg. The areas exposed were 0.43 square cm., and biopsy specimens of the exposed areas were taken 24 hours after exposure. Results from both experiments were similar. The radio-autographs showed that the lewisite was concentrated primarily in epidermis with very little in the dermis (Fig. 2). Unlike mustard, from which no visible injury occurred, the radio-activity in the epidermis was confined almost exclusively to dead cells. In contrast to the mustard experiments, there was a massive necrosis of most of the epidermal layer and the corium. This is probably to be explained by the fact that the effect of lewisite is more rapid on the skin than mustard, and the injury is detectable within 24 hours. The small amount of lewisite present in the corium was found to occur in regions of perivascular exudate (Figs. 2 and 3), in some, but not all, of the hair follicles (Fig. 2), and in some blood vessels (Figs. 2 and 3). The hair follicles accumulated the lewisite to about the same degree as epidermis. In one case in which involvement of the hair follicles occurred, activity was present in an associated sebaceous gland (Fig. 2).

Studies with Mustard on Pig Skin

In the first studies with mustard applied on pig skin, the exposure was for 6 hours to 2 mg. of the material, and the exposed areas were excised 24 hours after application. Results showed that mustard concentration was greatest in the epidermis, a slightly smaller amount in the corium, and very little in the hypodermis. The small amount of mustard in the hypodermis was found principally in the bands of

fibrous tissue surrounding the fatty tissue and in the deep blood vessels. A considerable amount of mustard was noted in the hair follicles and adjoining sebaceous glands (Figs. 4 and 5). The radio-autograph in Figure 4 indicates the variety of deep structures that have accumulated the material. A relatively large amount of mustard appears in the fibrous structure approximately midway between the epidermis and muscle which is apparently a fascial plane. Below this layer of fascia and a short distance above the muscle tissue, two blood vessels have accumulated a considerable amount of mustard. A very large proportion of the total amount retained was present in the epidermis. The section in Figure 5 represents a higher magnification of the region which has been delineated in Figure 4. The deposition in sebaceous glands, subcutaneous fibrous tissue, and blood vessels can be seen in somewhat better detail here.

A 15 minute exposure to 475 μg. of mustard gas, with immediate excision of the pig skin, showed much less penetration of the corium as compared with the 6 hour exposure to 2 mg. The mustard penetrated downward as far as the papillary layer of the dermis, and superficially located hair follicles accumulated mustard (Fig. 6). The 15 minute exposure with excision 24 hours later showed penetration into the dermis with accumulation in hair follicles well below the epidermis (Fig. 7).

Studies with Lewisite on Pig Skin

Two specimens of formalin-fixed pig skin contaminated with lewisite were studied. Sample 1 was exposed to 1.269 mg. of lewisite for 15 minutes, 24 hours prior to excision. Sample 2 was exposed to 1.534 mg. of lewisite for 1 hour, 24 hours prior to excision. It is apparent from viewing the photomicrographs of the skin sections and their corresponding radio-autographs that the distribution of lewisite is somewhat different than was noted with mustard. The labeled material is deposited primarily in the hair and hair follicles, with a smaller amount in the epidermis. Sebaceous glands and deeper structures of the skin, such as muscle, blood vessels, and corium, appear to accumulate negligible amounts of the labeled material as compared with hair and hair follicles.

The section in Figure 8 was taken from a sample of tissue to which the lewisite had been applied for a period of 15 minutes before being washed off. In the radio-autograph in Figure 8, the bulk of the activity appears to be concentrated in the hair follicles and shaft with very little present in the epidermis, a very faint wavy line indicating the accumulation of a small amount in the epidermis. The left-hand corner of the section in Figure 8 reveals a hair follicle and developing hair lying obliquely; the corresponding radio-autograph indicates that a

small amount of material was accumulated in the central portion of the follicle which, presumably, is occupied by the shaft of the hair. Figure 9 was taken from a section of tissue on which lewisite had been allowed to remain for 1 hour before its removal. The general appearance of the radio-autograph is similar to that noted for the 15 minute exposures, in that the bulk of the labeled material appears to be concentrated in the epidermis, hair, and hair follicles. Figure 9 reveals a deposition of the labeled material very clearly in the epidermal layer. The hair follicle in the center of the section apparently contained a very large amount of radio-arsenic, and, as a result, the radio-autograph gives a very dark blur. The follicles lying deeper in the sections have also apparently accumulated an appreciable amount of radio-arsenic.

Studies of Rabbit Eyes Exposed to Mustard Gas

Two experiments were done on rabbit eyes. In the first, liquid mustard was placed directly on the eye; in the second, the eye was subjected to the vapor by placing a small cup containing the material over the eye. The experiments were done at room temperature. Essentially similar results were obtained in both experiments, although the histologic preparations in the latter experiment were better and the interpretation of the radio-autographs thus facilitated. The results given here are based on the vapor-exposed eyes.

Figure 10 represents a 5 minute exposure to mustard, the eye having been removed immediately after exposure. The length of time required to bring out the small amount of activity in the lens and iris resulted in a loss in definition and gradient in the cornea. Figure 11 also represents a 5 minute exposure, but in this experiment the eye was not removed until the seventh day after exposure. It may be significant that the proportionate amount of activity present in the iris is greatly reduced in this case. This suggests the possibility that at least a portion of the activity shown in the iris (Figs. 10 and 11) may not represent fixed mustard but merely mustard or one of its derivatives which normally may pass through the iris into the blood stream. The alternative explanation that the mustard becomes "unfixed" more rapidly from iris tissue than from corneal tissue does not appear attractive.

Comparative Studies of Unfixed and Formalin-Fixed Tissues

For purposes of comparison with the results obtained from the formalin-fixed material and to rule out the possibility of migration of the two gases, radio-autographs were made from frozen, unfixed pig skin and human skin. These radio-autographs presented no significant

differences in appearance as compared to the radio-autographs from formalin-fixed sections.

Determination of Radio-Activity in Small Regions of Tissue Sections by Measuring the Darkening of the Photographic Films of Radio-Autographs

Through the technic of radio-autography, the deposition of a radio-active element in a tissue can be correlated with histologic structure. The radio-activity in a tissue as a whole can be easily measured by one of various counting devices, *i.e.*, Geiger counter, electroscope. On the other hand, the determination of the radio-activity of an individual structure has heretofore been extremely difficult. A method for determining the amount of activity in specific histologic structures has been worked out.

The degree of darkening of a radio-autograph is dependent upon the uptake of the radio-active element by the particular structure concerned. Since the darkening of photographic film can be measured by microphotometer tracings, it should be possible to determine the activity in a structure from the degree of darkening in the radio-autograph if standards of known activity producing a uniform darkening can be prepared for comparison. Since the radio-activity per mg. of mustard is known, the actual quantity of mustard in the histologic unit can be calculated assuming that the radio-active sulfur (S^*) atoms are still incorporated in the mustard molecules.

Standards, using BaS^*O_4, were prepared with shellac as a base for the radio-active salt. The following method was used:

(1) Solid BaS^*O_4 was mixed with thin shellac (about 1 mg. per cc. of shellac).

(2) The mixture was spread on glass slides with an oil paintbrush.

(3) To determine the uniformity of distribution of the BaS^*O_4, each slide was covered with photographic film, wrapped in black paper, and the package placed under a lead weight to hold the film in close contact with the slide. After 18 hours, the film was developed. Uniform areas of darkening were delineated by placing metal tubing of 1 cm. in outer diameter in the area and the remainder of the shellac on the slide scraped off. Agfa no-screen x-ray was used in these experiments; development was standardized at 2½ minutes at 21°C. in Eastman concentrated x-ray developer diluted 1:4.

(4) The activity of each sample was counted with a Geiger counter before exposure to the film. The film was exposed to samples for varying time intervals so that different degrees of darkening would be obtained. To determine the degree of darkening, or density, micropho-

tometer tracings were made of each film exposure. The following equation was used to express density:

$$D = \log_{10} \frac{i_o}{i}, \text{ where } i_o = \text{incident light, and } i = \text{transmitted light}$$

(5) Text-Figure 1 indicates graphically the relationship between the density and the total number of beta particles emitted by the
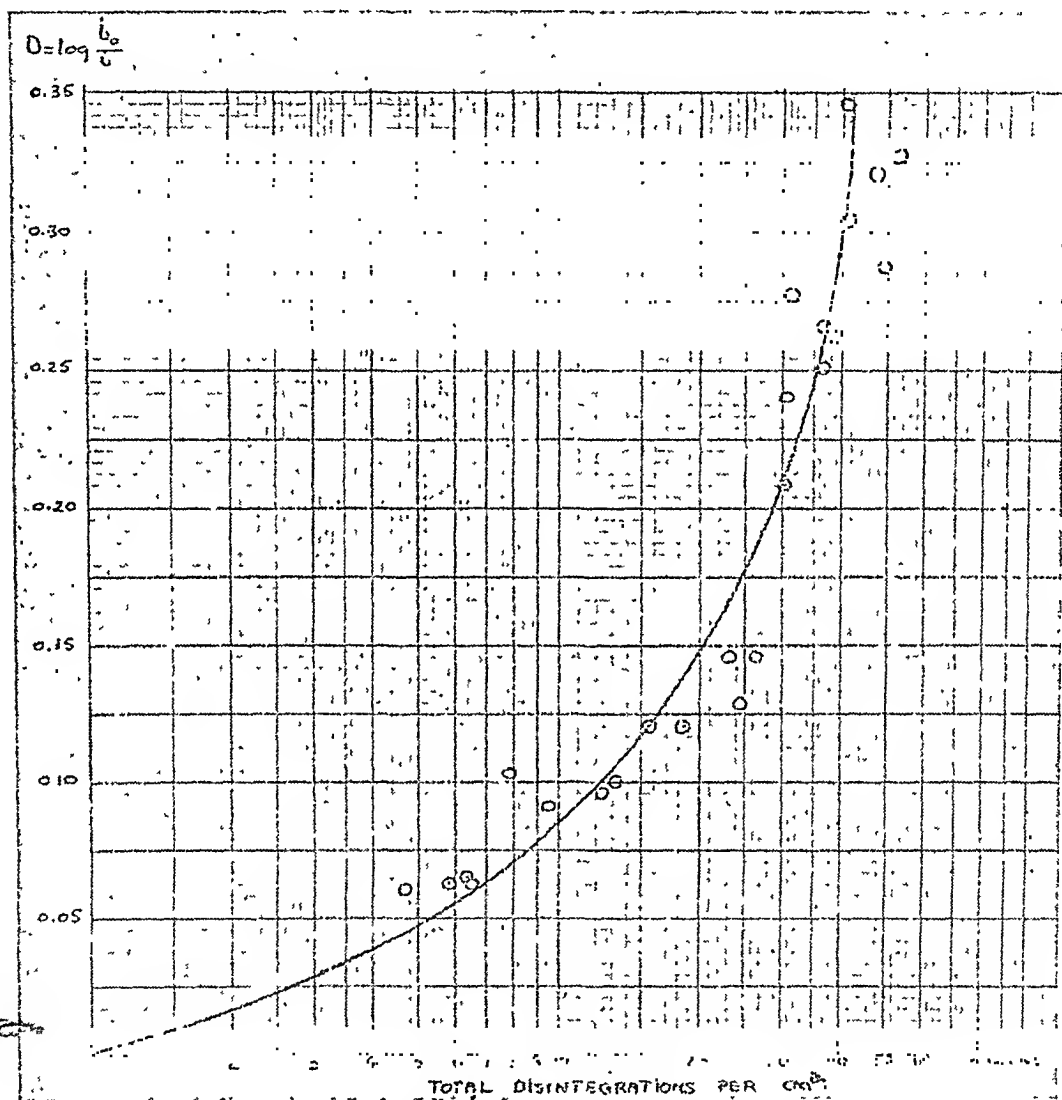
Text-Figure 1. Relationship between density of photographic blackening and the total number of disintegrations emitted by the sample.

sample. Corrections for the efficiency of the counter, decay of the radio-sulfur, and self-absorption of the beta particles from the radio-sulfur in the shellac film were made. It must be noted that the number of particles hitting the emulsion is only half of the total number of disintegrations minus those accounted for by self-absorption.

(6) Differences in the character of the emulsion, development, and in agitation during film development must be carefully controlled.

It is possible from the above data to estimate the quantity of radio-sulfur in a thin sample of radio-active material. This offers the possibility of employing microphotometric methods for measuring the darkening of film to determine the radio-activity present in small regions such as epidermis, hair follicles, sebaceous glands and blood vessels, thus leading to an estimate of the mustard content of a histologic unit. This interpretation assumes that radio-sulfur in the tissue is in the form of mustard and has not been split off and converted into other compounds. This procedure, is, of course, applicable to other radio-elements, but the calibration described in the above experiment would have to be repeated for the radio-element in question. This is due to the fact that the energies of the beta radiations differ for each radio-element, and the degree of darkening of the photographic emulsion varies with the energy of the radiation.

An experiment of this type can be illustrated as follows. A microphotometric tracing was made of the blood vessel located 3 cm. from the bottom of the photomicrograph in Figure 5. The density was found to correspond to a total number of disintegrations of approximately 20 million per square cm. The film was exposed to the tissue section for 14 days; thus the average, approximate activity in the blood vessel was 17 disintegrations per second during the interval in which the autograph was made.

The content of radio-sulfur per mg. of mustard was approximately 5 microcuries at the time the radio-autograph was made. This is equivalent to 1.9×10^5 disintegrations per second per mg. of mustard. Thus, 0.1 μg. of mustard was present in the 10 μ section of the blood vessel, the volume of the latter being 7×10^{-5} cmm., or, in other words, 14 mg. of mustard was present per gm. of blood vessel.

The tissue was exposed in such a manner that 1 mg. penetrated each square cm. of tissue. Of this 1 mg., about 250 μg. was fixed per square cm. of tissue, and 70 per cent of this fixed material was in the epidermis, which was 70 μ thick; thus the average mustard content of epidermis was 25 mg. per gm. Thirty per cent of the fixed material was in the corium, which is about 1.5 to 2 mm. thick; thus, the average mustard content of the corium was 0.37 mg. per gm.

SUMMARY AND CONCLUSIONS

The distribution of two war gases, mustard and lewisite, labeled with radio-active sulfur (S^{35}) and radio-active arsenic (As^{74}), respectively, in skin and eye tissues has been studied using the radio-autographic technic.

In human skin, lewisite was found to be fixed primarily in the epidermis, with very small amounts found in the dermis. The lewisite present in the dermis was found in some blood vessels, in regions of the perivascular exudate, in some hair follicles, and in one case in a sebaceous gland. There was massive necrosis of most of the epidermal layer and corium resultant from the lewisite.

Comparable studies with mustard gas applied on human skin showed this material to be fixed in epidermis and dermis. The blackening of the autographs was so great, due to the accumulation of mustard, that it was impossible to determine the specific concentration by blood vessels; hair follicles were seen so rarely that it was difficult to determine whether or not they fixed mustard. The marked degree of necrosis noted with lewisite gas was not apparent with the mustard gas; this may be explained by the fact that injury by mustard is not detectable within 24 hours, whereas the effect of lewisite is more rapid and necrotic effects are visible within a much shorter time.

A long exposure of pig skin to mustard (6 hours to 2 mg.) showed a high concentration in epidermis, dermis, hair follicles and adjacent sebaceous glands, and blood vessels. A small amount was found in the hypodermis in the bands of fibrous tissue surrounding fatty tissue, and also in deep blood vessels.

Shorter exposures of pig skin to mustard gas (15 minutes to 475 μg.) showed concentration in epidermis, dermis, and hair follicles.

Short exposures of pig skin to lewisite (15 minutes to 1.269 mg., and 1 hour to 1.534 mg.) showed concentration primarily in hair, superficially located hair follicles, and a very small amount in epidermis.

In all of the autographs, mustard gas penetrated the skin much more deeply than lewisite with a corresponding exposure. This deep penetration could explain the great destruction and deep burns resultant from exposure to mustard gas. In both the mustard and lewisite studies an accumulation of these two materials was noted in and around blood vessels. With destruction of blood vessels and subsequent local anemia one would expect slow healing of the affected skin area to ensue, which is a characteristic feature of these burns.

In rabbit eyes exposed to mustard gas this material was fixed primarily in the cornea, with a small amount in the conjunctiva, and a very small amount in the iris and lens.

By the method described it is possible to determine the radio-activity present in small regions of tissue sections, i.e., in blood vessels, hairs, hair follicles and accessory glands of the skin.

Ophthalmology, Harvard Medical School, with the eye studies; the generous cooperation of Dr. F. C. Henriques, Jr., and co-workers of the Gibbs Laboratory, Harvard University, in making available to us radio-active mustard and lewisite gas, and exposed skin.

BIBLIOGRAPHY

War Department Technical Manual: Treatment of Casualties from Chemical Agents. TM 8-285, July 10, 1941; TM 8-285, C-2, June 9, 1942.

Warthin, A. S., and Weller, C. V. The pathology of the skin lesions produced by mustard gas (dichlorethylsulphide). *J. Lab. & Clin. Med.*, 1918, 3, 447–479.

Winternitz, W. C. Collected Studies on the Pathology of War Gas Poisoning. Yale University Press, New Haven, 1920.

DESCRIPTION OF PLATES

PLATE 62

Fig. 1. Human skin exposed for 10 minutes to 475 µg. of mustard, tissue excised 24 hours after application. Fixation of mustard occurs in epidermis and corium. × 26.

Fig. 2. Human skin exposed for 15 minutes to 475 µg. of lewisite, tissue excised 24 hours after application. Fixation occurs primarily in epidermis, hair follicles (at extreme left of photomicrograph), and in an associated sebaceous gland (below follicles). The smaller squared area shows a blood vessel which has accumulated a small amount of activity. × 26.

PLATE 62

Lewisite and Mustard Gas in Skin and Eye
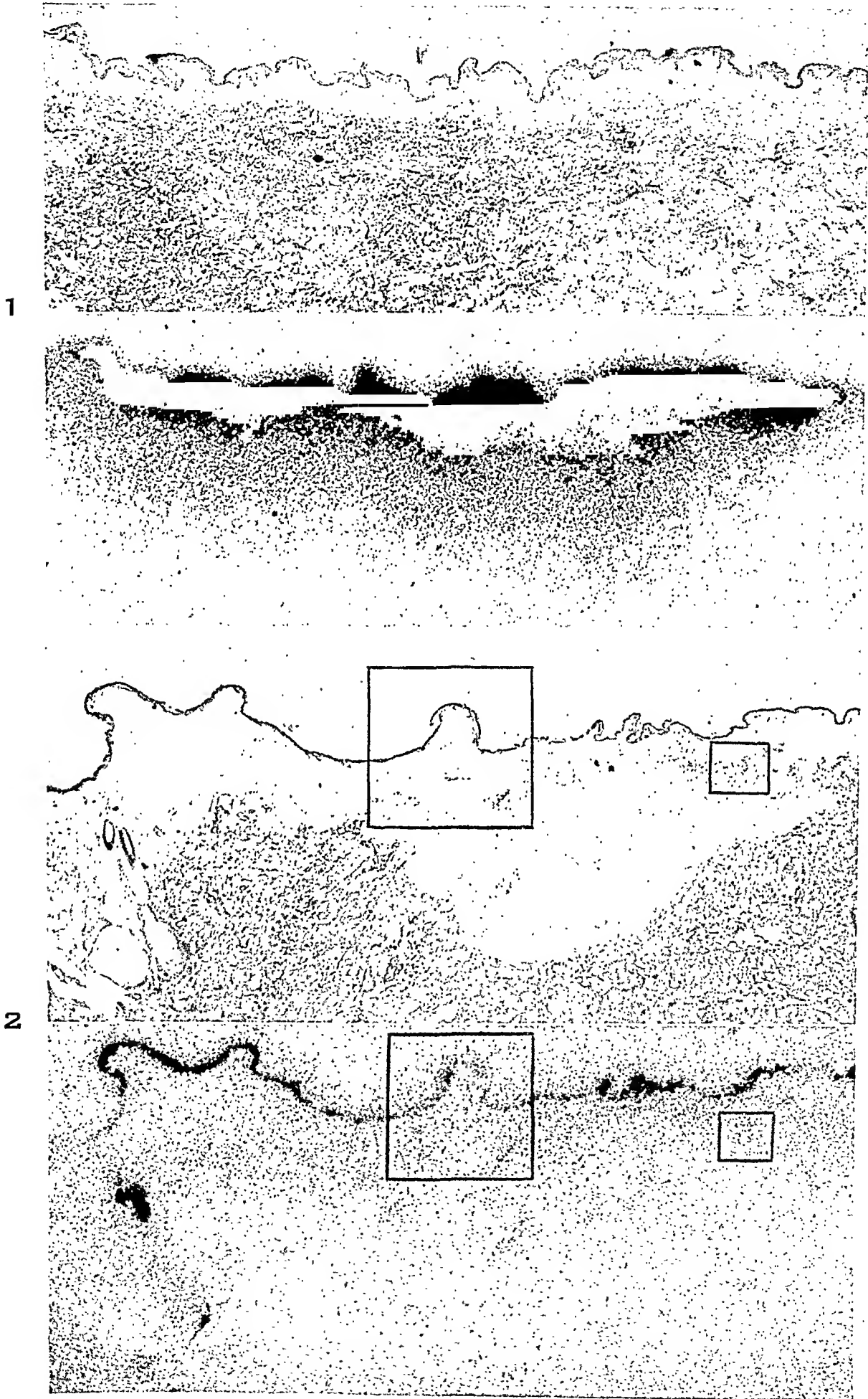
PLATE 63

FIG. 3. Human skin exposed to lewisite. Higher magnification of the larger squared area seen in Figure 2. Lewisite fixation occurs in the perivascular infiltration around blood vessels and in the epidermis separated from the papillary layer. × 100.

3
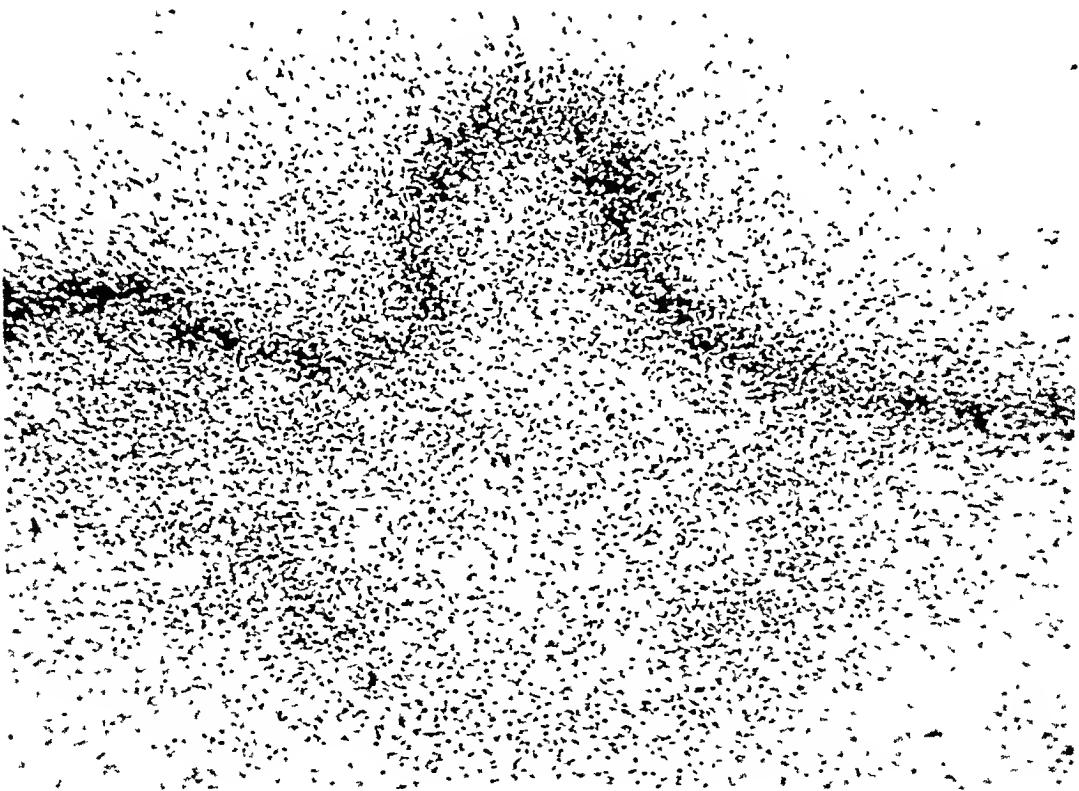
PLATE 64

FIG. 4. Pig skin exposed for 6 hours to 2 mg. of mustard and excised 24 hours after application. A large amount of mustard is fixed in the epidermis. corium, blood vessels. sebaceous glands, hair follicles, and subcutaneous fibrous tissues. × 10.

4

PLATE 65

FIG. 5. Pig skin exposed to mustard gas. Higher magnification of the squared area marked on Figure 4. Sebaceous glands (upper right), subcutaneous fibrous tissue (lower left), and blood vessels are seen to accumulate large amounts of mustard. X 30.

5

PLATE 66

FIG. 6. Pig skin exposed for 15 minutes to 475 μg. of mustard gas; tissue immediately excised. Most of the mustard fixation occurred in the epidermis, with negligible amounts extending into the papillary layer of the dermis. Superficially located hair follicles contained mustard. × 5.

FIG. 7. Pig skin exposed for 15 minutes to 475 μg. of mustard gas; tissue excised 24 hours later. Mustard has penetrated into the dermis, and is concentrated in deep-lying hair follicles. × 5.
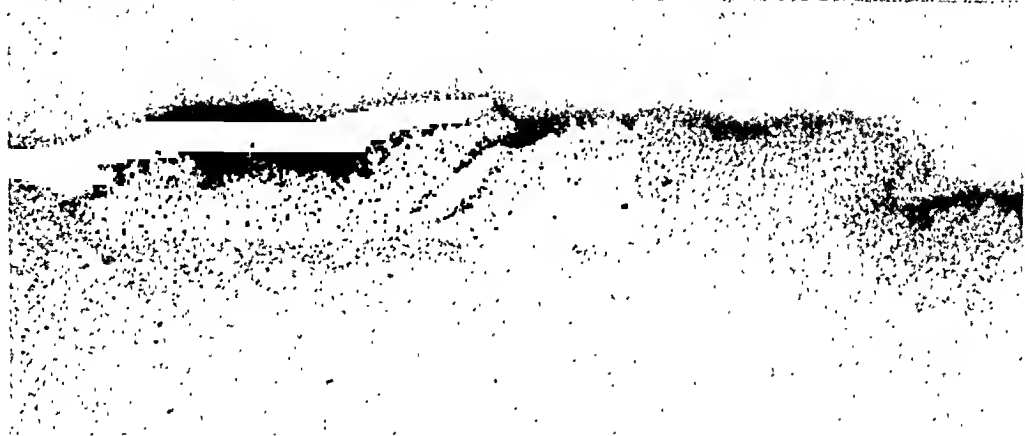
PLATE 67

FIG. 8. Pig skin exposed to 1.269 mg. of lewisite for 15 minutes; excision 24 hours later. The bulk of the lewisite is accumulated in hair follicles and hairs with smaller amounts in the epidermis. × 20.

FIG. 9. Pig skin exposed to 1.534 mg. of lewisite for 1 hour: excision 24 hours later. Lewisite has accumulated in hair, hair follicles, and epidermis. × 20.
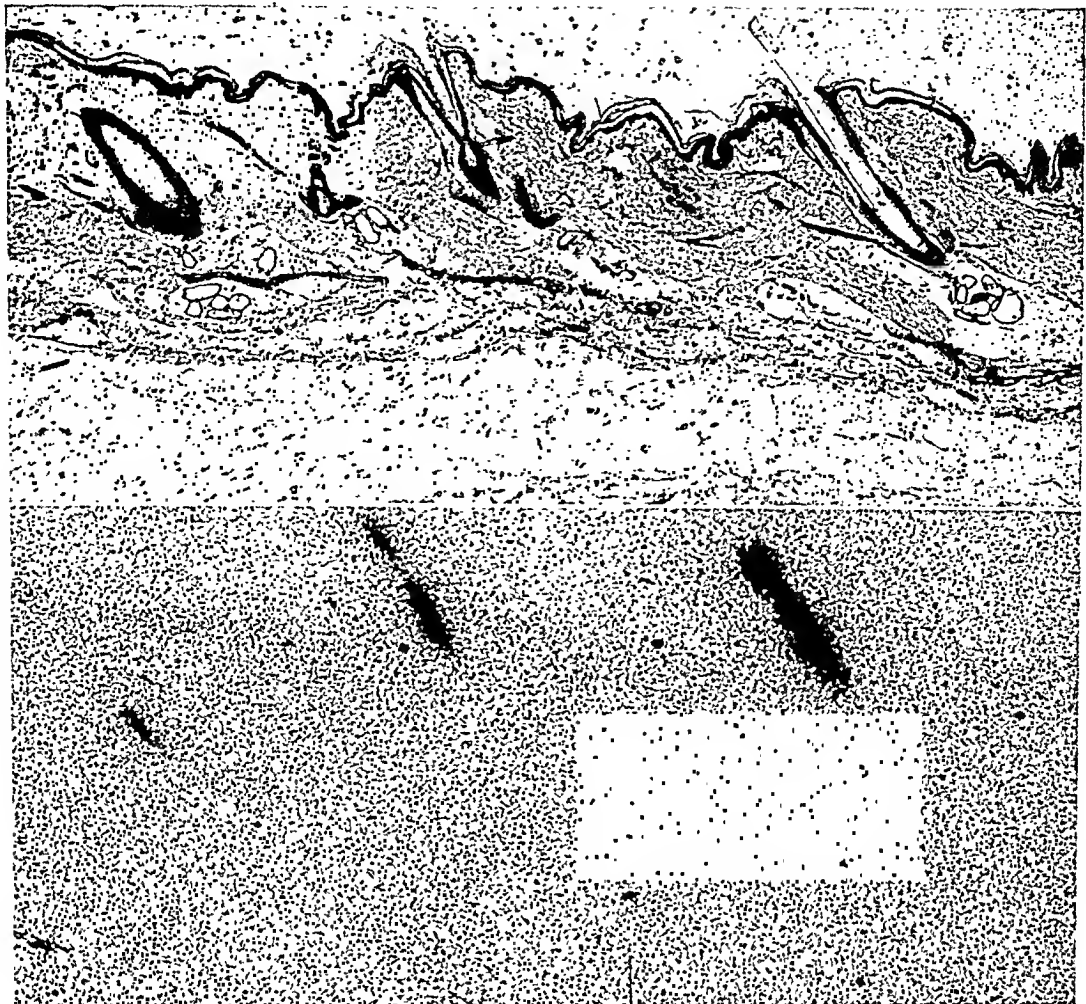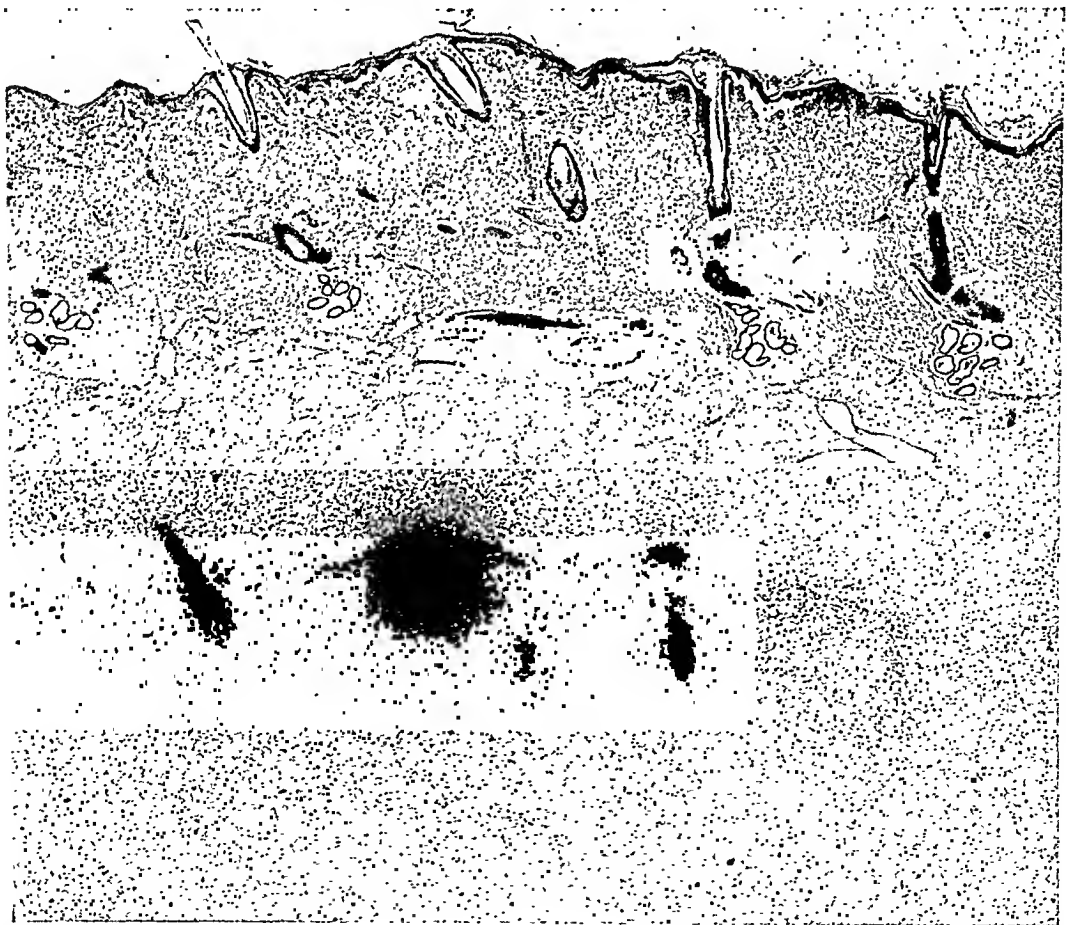
FIG. 10. Rabbit eye removed immediately after a 5 minute exposure to mustard vapor. Fixation is primarily in the cornea, with the iris, lens, and conjunctiva containing a lesser amount of activity. × 5.

FIG. 11. Rabbit eye removed 7 days after a 5 minute exposure to mustard. Fixation of mustard is primarily in the cornea. × 5.

PLATE 68

10

11
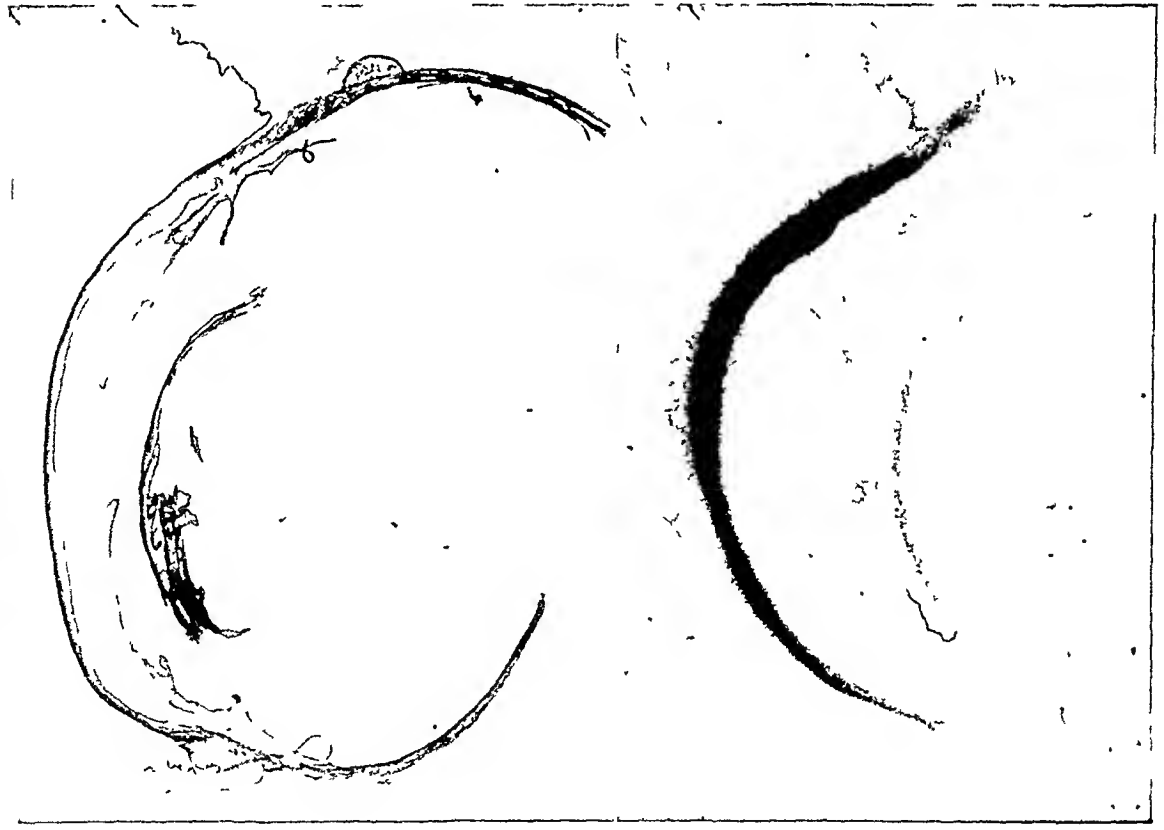
Axelrod and Hamilton
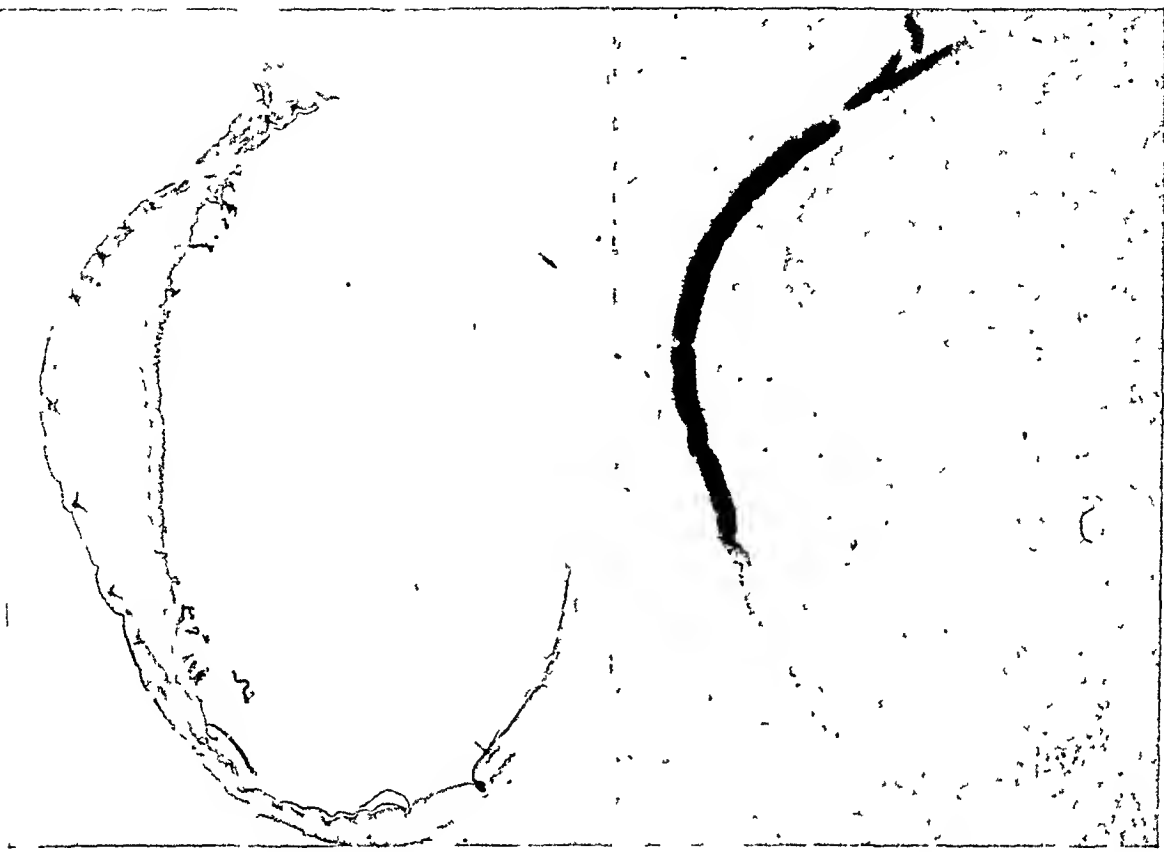
Lewisite and Mustard Gas in Skin and Eye

SO-CALLED PULMONARY ADENOMATOSIS AND "ALVEOLAR CELL TUMORS"

REPORT OF A CASE *

MORRIS A. SIMON, M.D.

(From the Department of Laboratories, Jewish General Hospital, Montreal, Quebec)

There occurs in man a relatively uncommon disease, so-called pulmonary adenomatosis, which bears a remarkable resemblance to an epizootic disease of sheep known variously as jaagsiekte, epizootic adenomatosis, pulmonary adenomatosis, verminous pneumonia, and Montana progressive pneumonia of sheep. This disease in sheep, which has been thoroughly described by Mitchell,[1] Dungal,[2] and Cowdry,[3] is characterized by marked inflammatory changes in the lung associated with an irregular proliferation of cells lining the alveoli. These cells adhere to the alveolar walls and, indeed, appear to arise from them. Such lining cells which histologically are epithelial in appearance vary from cuboidal to columnar, are not ciliated, show papillary infolding into the alveoli, and apparently are not continuous with bronchiolar epithelium. When fully developed the microscopic picture resembles an adenoma or adenocarcinoma but the cells are regular and metastases do not occur.†

In 1907 Helly[4] described an unusual tumor in man which resembles the condition found in sheep. Since that time 10 additional and "acceptable" cases occurring in man have been reported respectively by Löhlein,[5] Oberndorfer,[6] Bonne,[7] Richardson,[8] Breise,[9] Sims,[10] Bell,[11] Taft and Nickerson [12] (2 cases), and Wood and Pierson.[13] In all cases except one [13] the diagnosis was established at autopsy, as was true in the case reported here.

REPORT OF CASE

The patient was a white Jewish housewife, 70 years old, who was admitted to the medical service of Dr. David Mendel at about 10:30 a.m. on June 9, 1945, critically ill. The history, obtained from a daughter, stated that 7 weeks prior to admission the patient began to cough violently and to expectorate thick sputum which contained no blood. The coughing continued at night. During the week previous to admission the daughter had noted progressive dyspnea and orthopnea, and slight cyanosis. A general feeling of malaise and headache accompanied this condition. The past history revealed that the patient had had an attack of "bronchitis" the previous summer from which she apparently recovered. Otherwise, the past history and family history contained nothing contributing to knowledge of the present illness.

Physical examination revealed an emaciated, poorly developed patient showing moderate dyspnea and mild cyanosis of mucous membranes and nail beds. There

was dullness to percussion of both lung fields extending almost to the apices. Breath sounds could not be heard and moist, coarse, and bubbling râles were heard throughout the lungs. Heart sounds could not be heard due to the râles and the blood pressure was 150/75 mm. Hg. Other physical findings were within normal limits. The temperature, on admission, was 98.6°F.; pulse, 108 per minute; respiration, 42 per minute.

Within ½ hour after admission, the patient was placed in an oxygen tent and given coramine intravenously. Her respirations were labored. She was drowsy and took fluids very poorly. On the afternoon of admission, an electrocardiogram showed slight left axis deviation. A portable roentgenogram of the chest showed marked obscuration of both lung fields extending upwards to the level of the second rib anteriorly on the right and to the third rib anteriorly on the left. Both diaphragms and the cardiac silhouette were completely obscured. A small, translucent area in the left lower chest close to the outer aspect was noted.

Twenty-four hours after admission thoracentesis was attempted but no fluid could be aspirated. The patient's condition did not permit further laboratory studies. She died suddenly in the oxygen tent, 29 hours after admission.

The clinical diagnosis was pneumonitis, atypical, with bilateral pleural effusion.

POST-MORTEM FINDINGS

Autopsy was performed 17 hours after death.

The body was that of an emaciated, poorly developed adult white female, 70 years of age. Scarcely any recognizable subcutaneous fat was present. The right thoracic cavity was partially obliterated by dense, sheet-like adhesions which bound the lung in multiple areas to the chest wall. Where adhesions were not present, a fine deposit of granular, yellowish gray fibrin was noted on the pleural surfaces. On the left, similar but less extensive adhesions were present and the pleural surface of the left upper lobe contained a fine deposit of granular fibrin. The left lower lobe was small and collapsed, lay in a posterior position, and was flabby and almost cyst-like to palpation.

The upper third of the left upper lobe was crepitant and cottony in consistency, as was the lowermost portion of the left upper lobe. In the mid-portion of this lobe an irregular band of consolidated tissue was palpable. This band measured 8 cm. in width. Section revealed the upper and lower one-thirds of this lobe to be red and spongy while the middle one-third was gray, consolidated, slightly elevated, granular, and sticky (Fig. 1). Culture from this area yielded Friedländer's bacillus. The left lower lobe was small, violet in color, and collapsed like a bag when placed upon the table. Section revealed this entire lobe to consist of an irregularly loculated and trabeculated cavity 7 by 5 cm. in diameter. The wall of the cavity measured 2 to 3 mm. in thickness and it contained a small amount of yellowish gray, sticky, mucopurulent exudate. The bronchus to the left lower lobe communicated directly with this cavity and no alteration of the bronchial wall was noted.

On the right, all lobes were consolidated and noncrepitant. Section through all lobes revealed the major portion of each to consist of gray, granular, elevated, consolidated, noncrepitant tissue from which a considerable amount of sticky, yellowish gray exudate could be scraped. Culture from these lobes yielded Friedländer's bacillus and the gross appearance was that of lobar pneumonia in the stage of gray hepatization. No nodules of any type were present.

The major bronchi were filled with a large amount of sticky, yellowish gray, mucopurulent material. The mucous membranes were hyperemic but otherwise unaltered. The mediastinal lymph nodes were slightly enlarged and on section showed a mottled gray and black, homogeneous, moist surface. No tumor nodules of any type were present.

The balance of the gross examination failed to show any evidence of disease directly related to the lung condition other than cloudy swelling of the parenchymatous organs. There was generalized arteriosclerosis. A hemangioma of the liver and cystitis were present.

To summarize, the gross findings in the lungs were indistinguishable from ordinary pneumonia due to Friedländer's bacillus or Type III pneumococcus and of essentially lobar distribution, with an extensive remote abscess of the left lower lobe.

Microscopic Findings

Multiple sections taken from all lobes on the right revealed the majority of alveoli to be filled with large numbers of polymorphonuclear leukocytes, variable amounts of fibrin, and large numbers of pale, circular, phagocytic cells containing abundant, finely foamy cytoplasm and relatively small, dense, eccentrically placed nuclei (Fig. 2). Innumerable alveoli were lined either completely or partially by single and at times pseudostratified layers of remarkably tall-columnar epithelial cells (Fig. 3). These cells contained abundant, foamy cytoplasm staining pale pink with eosin and showed numerous goblet formations (Fig. 4) and brush borders of the free edge (Fig. 5). No cilia could be identified. The nuclei of these cells were oval, somewhat vesicular in character, and occasionally contained prominent nuclei. In many situations cells were apposed to the alveolar wall and, indeed, appeared to arise from the alveolus proper. In others these cells had become detached from alveolar walls and lay within alveolar lumina. Papillary infolding of these cells was a common occurrence. Only an occasional mitotic figure was encountered and the cells were remarkably uniform in size, shape, and staining quality.

The distribution of abnormally lined alveoli was quite irregular

throughout all sections (Fig. 6). Areas were encountered where practically all alveoli were lined by these cells, and in other fields there were no lining cells. No invasion of lymphatic or vascular spaces or of the pleura had occurred, although alveoli lying immediately adjacent to the pleura were lined by such cells.

Sections through the left upper lobe showed essentially the same picture as that seen in all lobes on the right. Sections taken through the thin wall of the cystic left lower lobe showed a trabeculated wall of condensed, anthracotic fibrous connective tissue diffusely infiltrated by lymphocytes and containing many thick-walled blood vessels. An occasional alveolus still remained and was lined by tall-columnar cells similar to those described above.

Sections through multiple hilar lymph nodes failed to reveal evidence of metastases, and the balance of the microscopic examination of organs failed to reveal changes relating to the pulmonary lesion.

DISCUSSION

From a clinical point of view, as far as can be determined, there are no pathognomonic signs, symptoms (except dyspnea), or roentgenologic appearances which would enable the clinician to make the ante-mortem diagnosis of pulmonary adenomatosis with any degree of certainty. The majority of these cases reported in the literature have been variously diagnosed clinically as pneumonia or tuberculosis, and, in some cases, tumors have been suspected. In the present case a clinical diagnosis of atypical pneumonia with abscess formation was made.

The gross appearance of the lungs in cases of human pulmonary adenomatosis has been described as resembling noncaseating miliary tuberculosis,[13] nodular and consolidated;[10] but in most cases has resembled lobar pneumonia in the stage of gray hepatization.[11,12] In none of the reported cases has any bronchial or bronchiolar lesion suggesting primary bronchiogenic carcinoma been described. In the cases of Taft and Nickerson [12] and in the present instance a considerable amount of sticky, gelatinous exudate could be scraped from the cut surface, suggesting a Friedländer's bacillus pneumonia.

Microscopically, the picture described by all authors is remarkably uniform, varying only with the degree of inflammatory change and extent of adenomatosis present. Irregularly scattered, large, diffusely involved areas show alveoli which are lined by either regular tall-columnar or high-cuboidal, nonciliated epithelial cells. These cells are remarkably uniform in size, shape, and staining quality and only rare mitotic figures are encountered. The nuclei of these cells are, for the most part, basally arranged, but at times lie in the midportion of the

cells. The chromatin in the nuclei is coarse and occasional nucleoli are noted. The cytoplasm of these lining cells is eosinophilic, reveals goblet formation and is slightly granular, and at times exhibits a brush border. These lining cells do not resemble the ciliated cells of bronchioles and, indeed, are not continuous with them. The hyperplastic epithelial cells rest upon apparently unaltered or, at times, minimally thickened, alveolar walls and frequently project in papillary folds into the lumina of alveoli. Desquamation of these cells in single-celled sheets is not uncommon and it would appear that these cells are very delicately attached to the alveolar walls. There is no destruction of alveolar walls and no lymphatic invasion is noted. Occasional mononuclear phagocytes with finely foamy cytoplasm are seen within alveoli and the amount and character of the exudate within alveoli are variable.

Attempts to stain for mucin in the present case were uniformly unsuccessful, but Taft and Nickerson [12] noted mucin which stained with aniline dyes.

The similarity between human pulmonary adenomatosis and the lesions occurring in sheep raises a number of interesting speculations. While no specific etiologic agent has been proved as the cause of jaagsiekte in sheep, the work of Dungal, Gislason, and Taylor [14] strongly suggests that the disease is infectious and communicable. A virus has been suggested by Cowdry,[3] Bonne,[7] and Bell,[11] but attempts to demonstrate a causative agent and/or to transmit the disease to laboratory animals have been unsuccessful. Amongst sheep, however, the transmission can easily be effected by housing sheep together with one or more diseased animals. In only one instance were Dungal, Gislason, and Taylor able to transmit the disease from one sheep to another by intrapulmonary inoculation. There is, at present, no evidence to suggest that pulmonary adenomatosis in man is of an infectious or communicable nature, but it must be remembered that too few observations have as yet been made to draw any conclusions. Attempts to transmit the disease to various laboratory animals from human autopsy material by Richardson,[8] Sims,[10] and Wood and Pierson [13] have been uniformly unsuccessful.

Another interesting problem which pulmonary adenomatosis raises is the controversial question regarding the presence and nature of alveolar lining cells. On the one hand, Bensley and Bensley,[15] Miller,[16] and Cooper [17] have maintained that a continuous layer of epithelial cells line the alveoli, while Maximow and Bloom,[18] Rose,[19] Fried,[20] and Loosli [21] have stated that the lining cells are mesenchymal. Ross [22] believes that both mesenchymal and epithelial cells line alveoli. While this complex problem remains to be settled definitively, the fact that the

cells lining the alveoli in human pulmonary adenomatosis appear to be multicentric in origin, appear to spring *de novo* from the alveolar walls, and are nonciliated and noncontinuous with the bronchiolar epithelium strongly suggests that cells do line alveoli either completely or incompletely. It is certain that in pulmonary adenomatosis these cells are of an epithelial nature. Whether these proliferating cells spring from pre-existing epithelial cells or represent metaplasia from an indifferent type of cell under varying pathologic conditions is difficult to state categorically. Oberndorfer,[6] Bell,[11] and Taft and Nickerson[12] believe these cells have an epithelial origin, and Bell believes that occasional epithelial lining cells may be found in post-natal lungs. Herbut[23] has attempted to demonstrate that the epithelial cells lining the alveoli are derived from bronchiolar epithelium. This concept does not account for the distinctly different appearance of the tall, nonciliated epithelial cells of pulmonary adenomatosis from that of the relatively low ciliated cells lining the bronchioles. Furthermore, since widespread bilateral pulmonary adenomatosis without lymphatic invasion does occur, the concept that this arises on the basis of metaplasia from bronchiectatic foci seems unlikely. In none of the reported cases, as in the present case, was bronchiectasis a feature. Geever, Neubuerger, and Davis,[24] on the other hand, while recognizing the presence of epithelium-like septal cells, refused to commit themselves as to the nature of the cell and referred to tumors arising from these cells as "alveolar cell" tumors. However, Helly's[4] opinion that the cells arise from alveolar duct epithelium cannot be entirely dismissed.

The question whether human pulmonary adenomatosis, as the name suggests, is an entirely benign, hyperplastic (*i.e.*, nonmetastasizing) process or whether it may be the initiating point for some of the so-called alveolar tumors described and collected by Neubuerger and Geever[25] is of considerable importance. Of the 12 "acceptable" cases (including the one reported here), only those of Oberndorfer[6] and Breise[9] showed metastases to lymph nodes. All other cases not only appeared to be benign from a histologic standpoint but failed to show metastases. The only instance of metastasis in jaagsiekte of sheep appears to be that of Aynaud, Peyron, and Falchetti (cited by Dungal[2]) in which a metastasis was found in a regional peribronchial lymph node. It appears, therefore, that occasional cases of jaagsiekte and pulmonary adenomatosis may, and indeed do, metastasize, thus fulfilling all the criteria of a malignant neoplasm.

In no instance in the reported cases of pulmonary adenomatosis has there been demonstrated a primary bronchial focus which would fulfill the recognized criteria. The fact that the disease is usually bilateral

without any histologic evidence of lymphatic invasion strongly suggests that it is multicentric in origin. Under these circumstances, it seems reasonable to regard pulmonary adenomatosis as a well differentiated, relatively slowly growing but eventually metastasizing pulmonary tumor of an unusual type which differs in the details mentioned above from the ordinary bronchiogenic carcinoma.

Neubuerger and Geever [25] collected from the literature 43 cases of unusual tumors of the lung to which they gave the name "alveolar cell tumors" in order to avoid controversy as to the histogenesis of the cells. Included in this group are the "acceptable" cases of so-called pulmonary adenomatosis of Helly,[4] Löhlein,[5] Oberndorfer,[6] Bonne,[7] Richardson,[8] and Breise.[9] These cases differ in no significant respects from the remainder of the 43 collected cases, all of which showed a pathologic change indistinguishable from pulmonary adenomatosis. Of these 43 cases, 24 (or 56 per cent) showed metastases. All of the collected cases of Neubuerger and Geever, whether controversial or otherwise, in their opinion, showed characteristic cuboidal to columnar epithelial cells with or without papillary infolded lining alveoli, and in all cases the usual bronchiogenic origin was excluded. No evidence that the lung lesions were metastatic in nature was present. In 1945 Geever, Carter, Neubuerger, and Schmidt [26] reported 6 additional cases of "alveolar cell" tumor. In 4 of these, metastases were present and showed characteristic cuboidal or columnar cells lining alveoli; in all, the usual bronchiogenic type of tumor was excluded.

It would seem, therefore, that pulmonary adenomatosis and "alveolar cell" tumors are, from a cytologic point of view, identical and that the presence and extent of metastases are variable. It may well be that the degree of pneumonia which usually accompanies this disease in both sheep and man may cause death before metastases develop.

SUMMARY

A case of histologically benign pulmonary adenomatosis without metastases forms the basis of this report. This condition is similar to the epizootic disease of sheep. In occasional cases of jaagsiekte and of pulmonary adenomatosis metastasis has occurred.

Cytologically, the cases of so-called pulmonary adenomatosis appear to be identical with a much larger series of collected and reported cases which have been termed "alveolar cell" tumors, about half of which metastasize.

Pulmonary adenomatosis and alveolar cell tumors may be regarded as unusual forms of pulmonary carcinoma presumably arising from alveolar lining cells.

Since this paper was submitted for publication, Dungal (*Am. J. Path.*, 1946, 22, 737–759) has reported upon his experiences with experimental jaagsiekte. He concluded that jaagsiekte is due to a pneumotropic virus strictly limited to the lungs and bronchi of sheep and excreted with the respiratory air. He stated that no cases of pulmonary adenomatosis have appeared among shepherds who are in contact with sick sheep for long periods of time and he therefore believes that man is immune to this particular virus.

Also, since submitting this paper, I have had an opportunity to study by autopsy an additional case of "alveolar cell" tumor of the lungs. In this case the lesions in the lungs are identical with those reported. No metastases were present in the broncho-pulmonary or mediastinal lymph nodes but metastases were found in the brain. This study emphasizes the belief that pulmonary adenomatosis and "alveolar cell" carcinomas of the lung are probably identical.

REFERENCES

1. Mitchell, D. T. Investigations into jaagsiekte or chronic catarrhal-pneumonia of sheep. *Union So. Africa, Dept. Agric., Rep. of Dir. of Vet. Research,* 1915, 3 and 4, 585–614.
2. Dungal, N. Epizootic adenomatosis of the lungs of sheep: its relation to verminous pneumonia and jaagsiekte. *Proc. Roy. Soc. Med.,* 1937–38, 31, 497–505.
3. Cowdry, E. V. Studies on the etiology of jaagsiekte. I. The primary lesions. *J. Exper. Med.,* 1925. 42, 323–333. Cowdry, E. V., and Marsh, H. Comparative pathology of South African jaagsiekte and Montana progressive pneumonia of sheep. *J. Exper. Med.,* 1927, 45, 571–585.
4. Helly, K. Ein seltener primärer Lungentumor. *Ztschr. f. Heilk.,* 1907, 28, 105–110.
5. Löhlein, M. Cystisch-papillärer Lungentumor. *Verhandl. d. deutsch. path. Gesellsch.,* 1908, 12, 111–115.
6. Oberndorfer, S. Zellmutationen und multiple Geschwulstentstehungen in den Lungen. *Virchows Arch. f. path. Anat.,* 1930, 275, 728–737.
7. Bonne, C. Morphological resemblance of pulmonary adenomatosis (jaagsiekte) in sheep and certain cases of cancer of the lung in man. *Am. J. Cancer,* 1939, 35, 491–501.
8. Richardson, G. O. Adenomatosis of the human lung. *J. Path. & Bact.,* 1940, 51, 297–298.
9. Breise. Zur Kenntnis des primären Lungenkarzinoms, mit statistischen Angaben. *Frankfurt. Ztschr. f. Path.,* 1920, 23, 48–55.
10. Sims, J. L. Multiple bilateral pulmonary adenomatosis in man. *Arch. Int. Med.,* 1943, 71, 403–409.
11. Bell, E. T. Hyperplasia of the pulmonary alveolar epithelium in disease. *Am. J. Path.,* 1943, 19, 901–907.
12. Taft, E. B., and Nickerson, D. A. Pulmonary mucous epithelial hyperplasia (pulmonary adenomatosis). A report of two cases. *Am. J. Path.,* 1944, 20, 395–403.
13. Wood, D. A., and Pierson, P. H. Pulmonary alveolar adenomatosis in man. Is this the same disease as jaagsiekte in sheep? *Am. Rev. Tuberc.,* 1945, 51, 205–224.
14. Dungal, N., Gislason, G., and Taylor, E. L. Epizootic adenomatosis in the lungs of sheep. Comparisons with jaagsiekte, verminous pneumonia and progressive pneumonia. *J. Comp. Path. & Therap.,* 1938, 51, 46–68.
15. Bensley, R. D., and Bensley, S. H. Studies of the lining of the pulmonary alveolus of normal lungs of adult animals. *Anat. Rec.,* 1935–36, 64, 41–49.
16. Miller, W. S. The Lung. C. C. Thomas, Springfield, Ill., 1937.

17. Cooper, E. R. A. A histologic investigation of the development and structure of the human lung. *J. Path. & Bact.*, 1938, 47, 105–114.
18. Maximow, A., and Bloom, W. A. A Textbook of Histology. W. B. Saunders Co., Philadelphia, 1930.
19. Rose, S. B. The finer structure of the lung with special reference to its vascular character and its pathologic significance. *Arch. Path.*, 1928, 6, 36–47.
20. Fried, B. M. Primary carcinoma of the lung. Bronchiogenic cancer—a clinical and pathological study. *Medicine*, 1931, 10, 373–508.
21. Loosli, C. G. The structure of the respiratory portion of the mammalian lung with notes on the lining of the frog lung. *Am. J. Anat.*, 1937–38, 62, 375–425.
22. Ross, I. S. Pulmonary epithelium and proliferative reactions in the lungs. A study of the cellular response in lungs after intratracheal injection of toxic and nontoxic foreign substances. *Arch. Path.*, 1939, 27, 478–496.
23. Herbut, P. A. Bronchiolar origin of "alveolar cell tumor" of the lung. *Am. J. Path.*, 1944, 20, 911–929.
24. Geever, E. F., Neubuerger, K. T., and Davis, C. L. The pulmonary alveolar lining under various pathologic conditions in man and animals. *Am. J. Path.*, 1943, 19, 913–937.
25. Neubuerger, K. T., and Geever, E. F. Alveolar cell tumor of the human lung. *Arch. Path.*, 1942, 33, 551–569.
26. Geever, E. F., Carter, H. R., Neubuerger, K. T., and Schmidt, E. A. Roentgenologic and pathologic aspects of pulmonary tumors probably alveolar in origin, with report of 6 cases, one of them complicated by torulosis of the central nervous system. *Radiology*, 1945, 44, 319–327.

[Illustrations follow]

DESCRIPTION OF PLATES

PLATE 69

Fig. 1. Left lung showing the character of the cut surface of the midportion of the left upper lobe. The left lower lobe contains the collapsed, cystic, remote abscess seen at the left.

Fig. 2. Pneumonic exudate consisting of fibrin, mucus, and large phagocytic cells with finely foamy cytoplasm as well as lymphocytes and rare polymorphonuclear leukocytes. Of note are the desquamated lining cells in the center of the field and the cuboidal cells lining the alveolar septa. Hematoxylin and eosin stain. $\times$ 215.
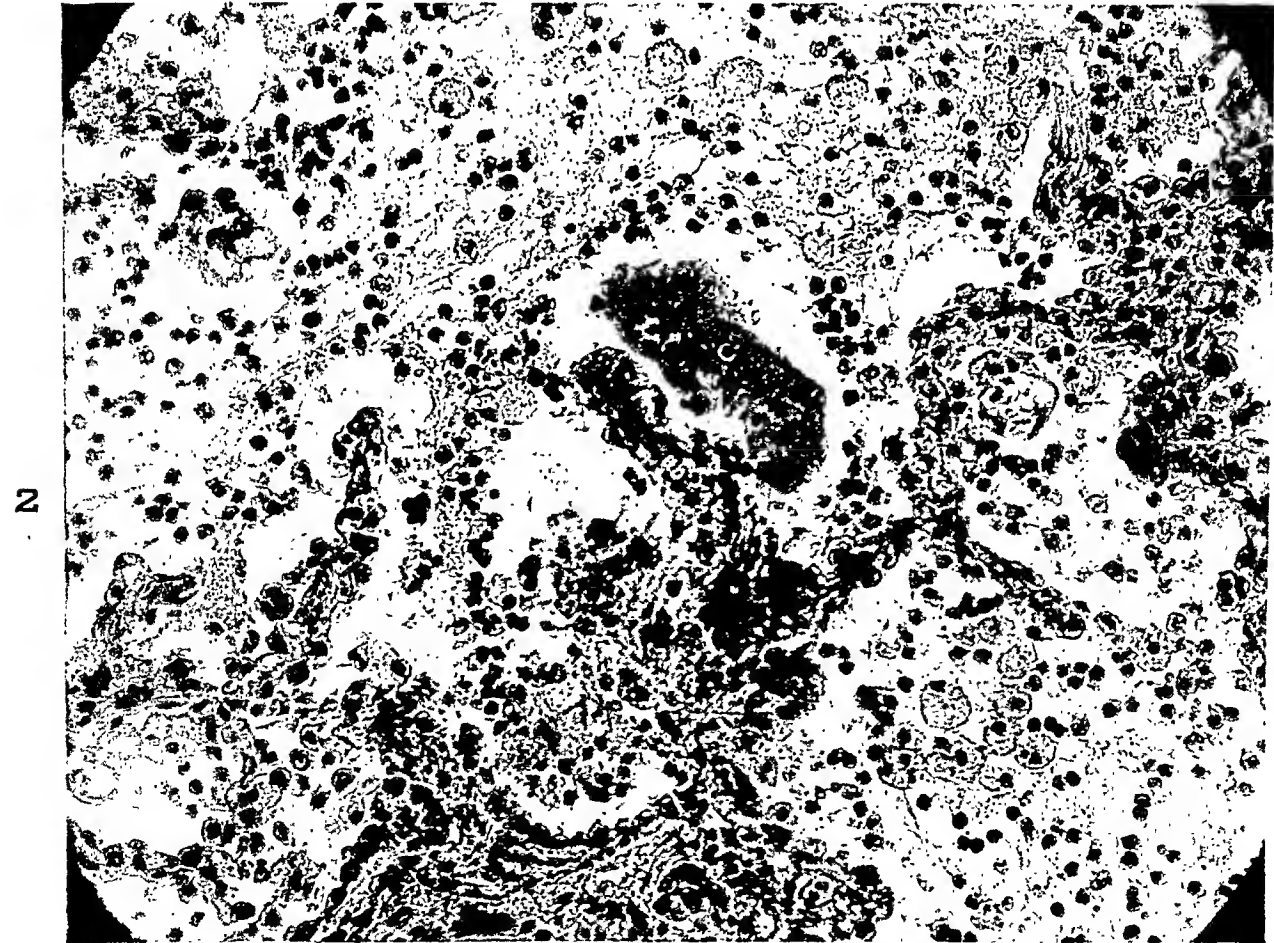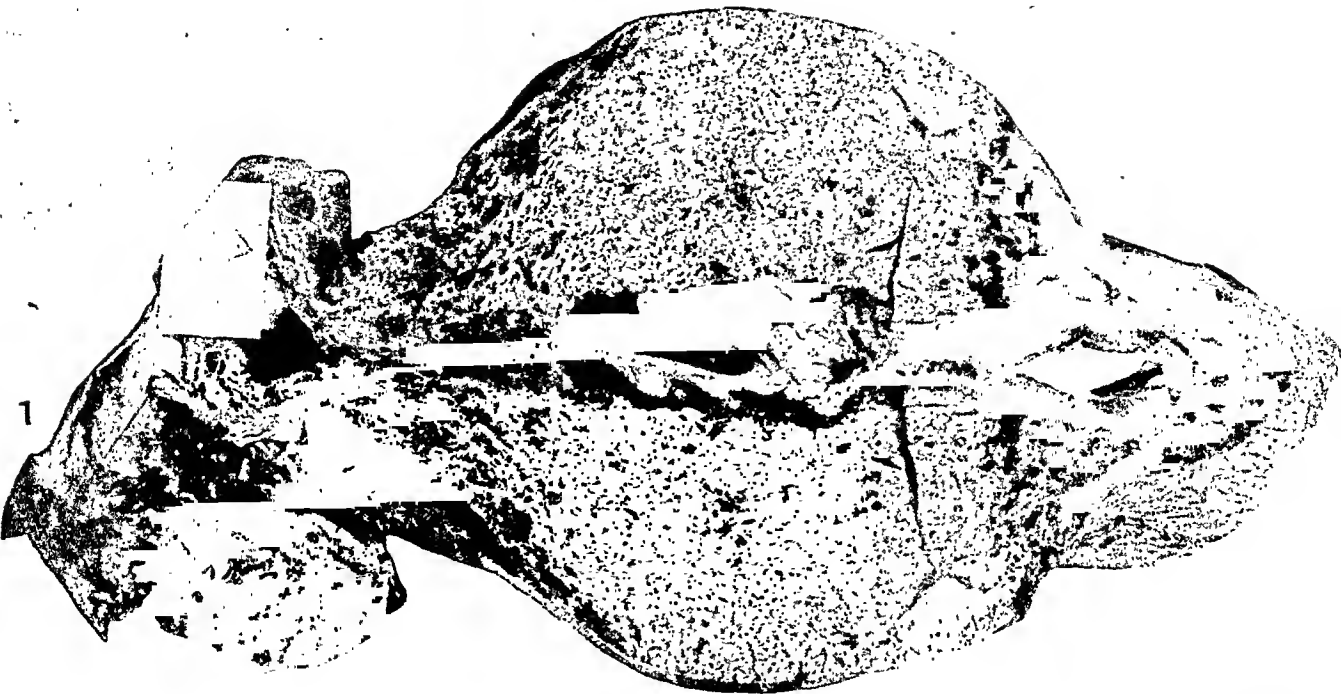
PLATE 70

FIG. 3. Appearance of alveoli lined by tall-columnar cells showing papillary infolding and goblet cells. Alveolar walls are thin, delicate, and unaltered. Hematoxylin and eosin stain. $\times$ 115.

FIG. 4. This photomicrograph demonstrates the delicate attachment of lining cells to the unaltered alveolar walls, together with the tendency toward detachment and desquamation of the cells into the lumen. Goblet formation is well seen here. Hematoxylin and eosin stain. $\times$ 210.
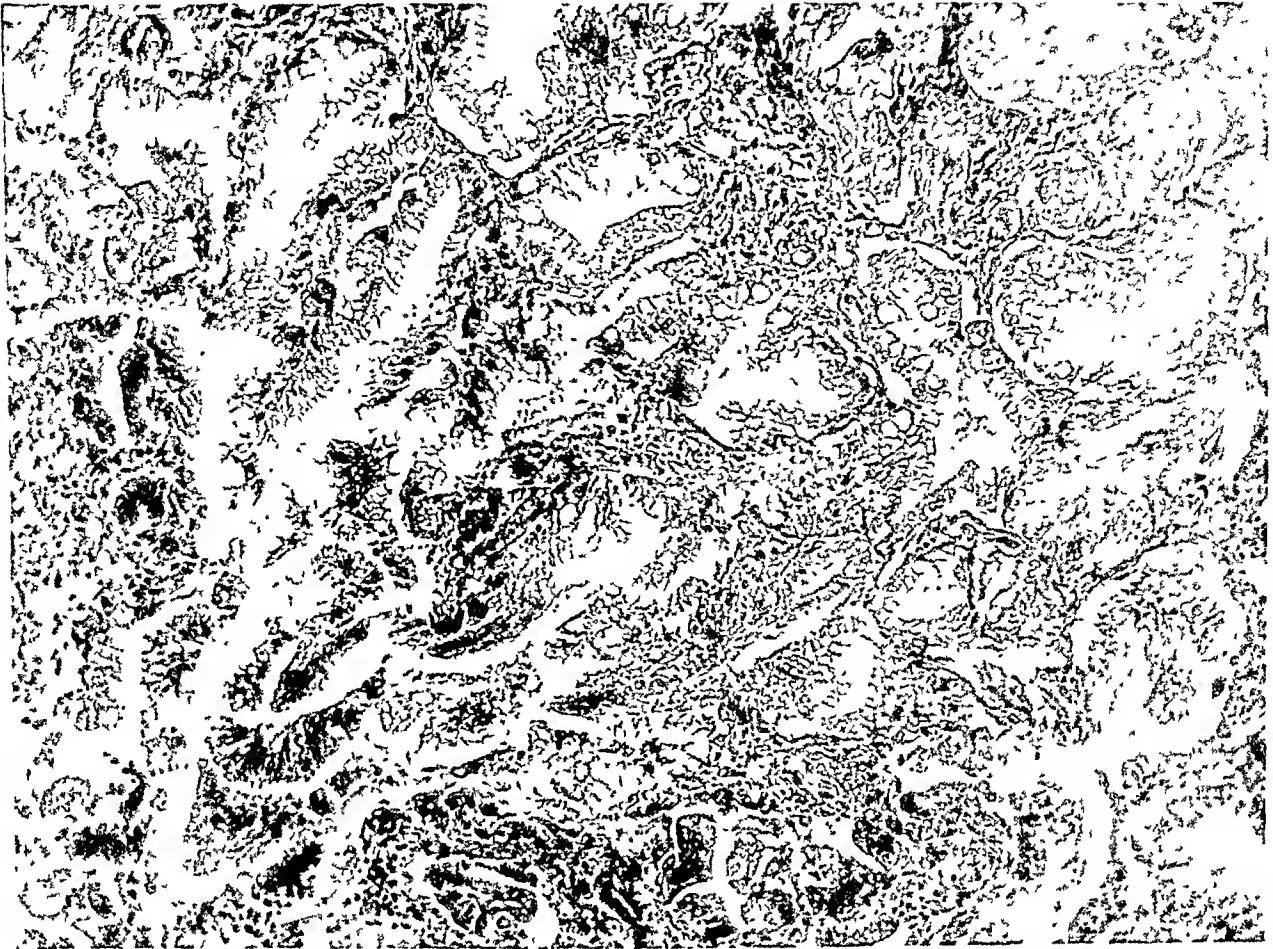
FIG. 5. Of note are the brush borders of cells lining the alveolus on the right and the character of the nuclei and cytoplasm. Hematoxylin and eosin stain. × 460.

FIG. 6. Low-power view showing the general distribution of pneumonia and of the neoplasm. Hematoxylin and eosin stain. × 23.

Plate 71

5
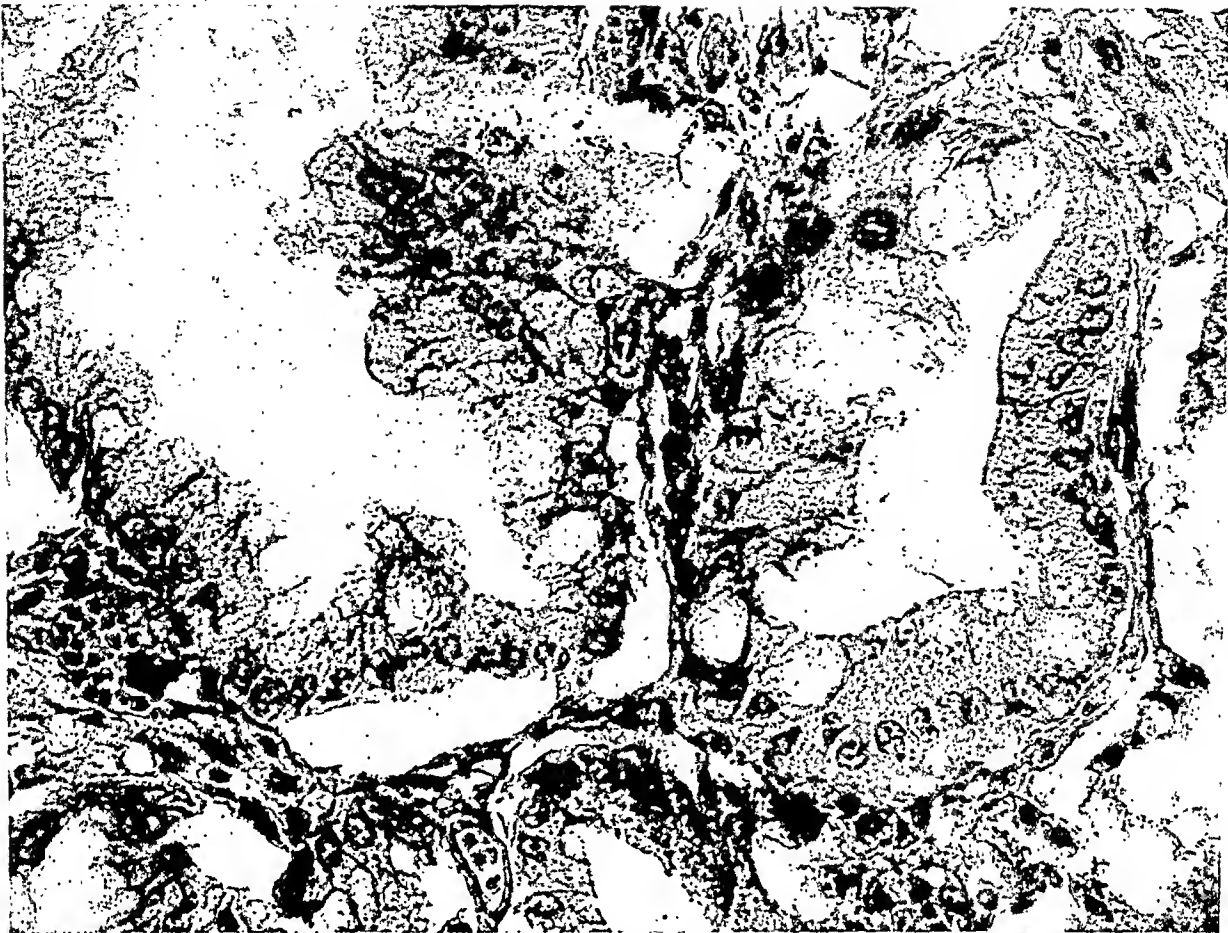
6
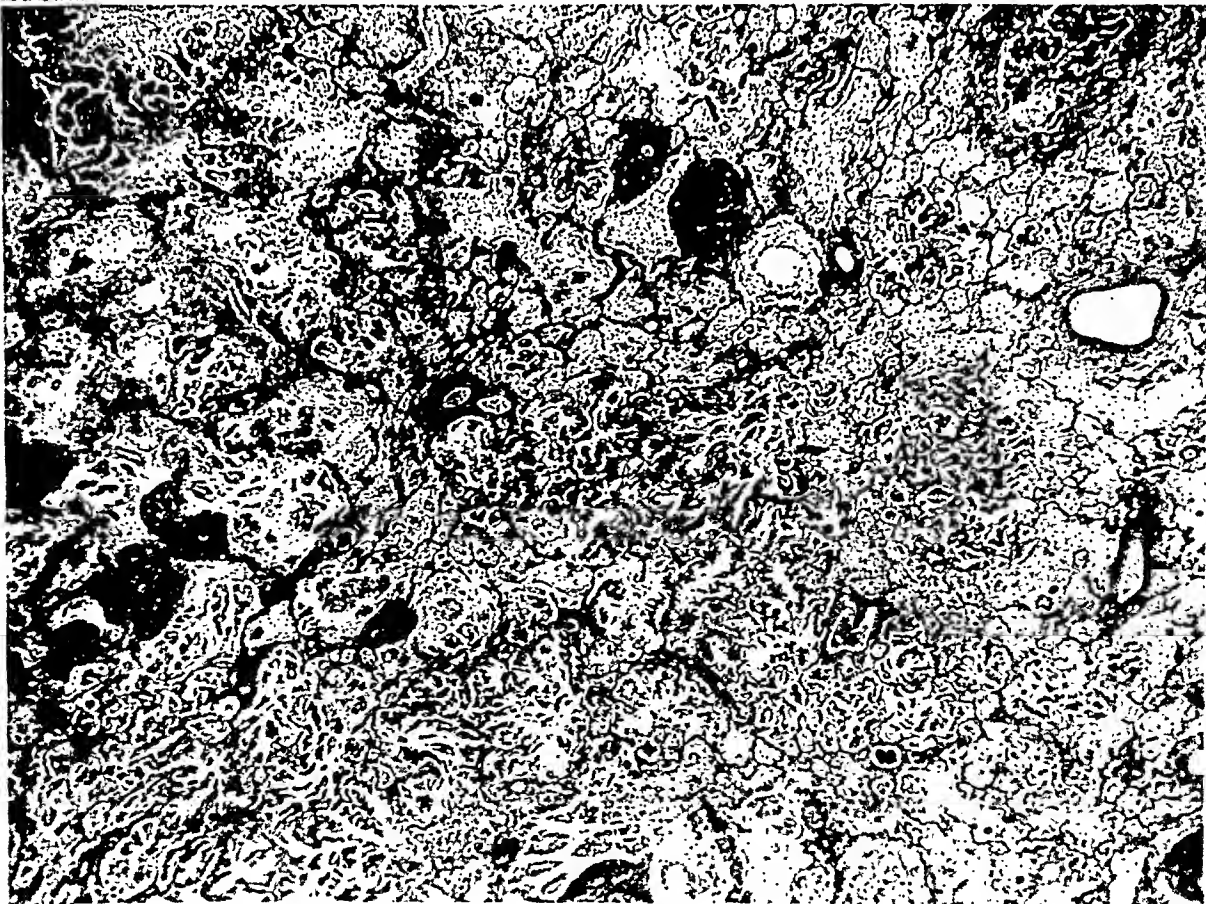
DISTINCTIVE CHARACTERISTICS OF THE SYMPATHICOBLASTOMA CULTIVATED IN VITRO

A Method for Prompt Diagnosis *

Margaret R. Murray, Ph.D., and Arthur Purdy Stout, M.D.

(*From the Surgical Pathology Laboratory of the College of Physicians and Surgeons, Columbia University, and the Department of Surgery of the Presbyterian Hospital, New York, N.Y.*)

The tumor here designated *sympathicoblastoma,* sometimes called *neuroblastoma,* is a highly malignant neoplasm composed of small sympathicoblasts which are often undifferentiated but sometimes arrange themselves in the form of rosettes or pseudorosettes. It is generally regarded as being derived from sympathetic nervous tissue, usually, although not always, arising in connection with a sympathetic ganglion in the mediastinal or retroperitoneal regions, or in the suprarenal medulla. Its chief incidence is among infants and young children, although it may be found in adults of various ages.

The observations here reported were gleaned in the course of a long-term study of the form and behavior of human tumors *in vitro,* during which eight sympathicoblastomas were investigated. These eight tumors behaved in a uniformly distinctive manner *in vitro;* their differences from other tumors with which they are likely to be confused clinically or histologically were so marked that we were led to use tissue culture as a diagnostic method for this tumor. By this means the diagnosis can now be made with a high degree of certainty in 24 hours or less.

Case Histories

Case 1

W. H. (S.P. 67384), an American boy, 3 years old, suffered for 3 months with symptoms due to metastases in the skull and elsewhere. When examined there were retroperitoneal masses and evidences of metastases throughout the skeleton, in the orbit, the scalp, and the abdominal wall. He died 6 weeks after admission to the hospital. Specimen taken for biopsy of one of the tumors in the scalp showed masses of undifferentiated sympathicoblasts with much hemorrhage and necrosis and no evidence of rosette or pseudorosette formation.

Case 2

Baby M (S.P. 73120), a male child of German-American parentage, was born with a large globular mass in the outer part of the left leg near the knee. After biopsy a mid-thigh amputation was done at the age of 1 month. Eighteen months later a retroperitoneal mass near the right kidney was explored and examined by biopsy. Roentgenotherapy was used over a period of 2 months but did not prevent the appearance of other metastases. When the child died at the age of 3⅓ years, autopsy showed metastases in the liver, right kidney, lungs, pleura, mesentery,

vertebrae, skull and humerus, from the retroperitoneal tumor. Microscopically, the tumor consisted of undifferentiated sympathicoblasts packed together tightly without the formation of either true or pseudorosettes.

Case 3

R. D. (S.P. 85876), an American male child, 12 months old, was found to have fluctuant swellings in both temporal regions and in the right thigh 1 week before admission. Tissue was taken for biopsy from the mass in the thigh and the child died 3 months later with extensive bone metastases and nodules in the abdominal cavity, pleurae, left lung, liver, and right testis. There was no autopsy but the primary site was believed to be retroperitoneal. Microscopically, the tumor was made up of sympathicoblasts which in some areas surrounded irregular spaces partly filled with tangled neurites. This represented partial differentiation. No perfectly rounded pseudorosettes were found.

Case 4

C. C. (S.P. 88130), a colored man, 33 years old, had noted a progressively increasing solitary swelling in the left groin. The mass was discrete, measured 8 by 5 cm., and was examined by biopsy. He received roentgenotherapy over a 1-month period. The field was 15 by 15 cm., later reduced to 10 by 10 cm. The factors were 190 kv., 10 ma., target skin distance 50, filter 1 mm. Cu, and a total dose of 3000 r. was given. As a result the tumor disappeared entirely and he was well 18 months after biopsy. Microscopically, the tumor was composed of solid masses of sympathicoblasts with extensive necrosis. Occasional pseudorosettes were present.

Case 5

H. F. (S.P. 88959), a boy, 6 years old, of Hungarian Jewish descent, complained of pain in the thoracic vertebrae for 6 months. Roentgenograms showed a mediastinal mass which at operation proved to be a lobulated, smooth, rubbery tumor, projecting into the right pleural cavity and pushing the trachea forward and the arch of the azygos vein downwards. It was impossible to remove the neoplasm completely and the child died 3½ months later. Microscopically, the tumor was made up of sympathicoblasts which formed a considerable number of pseudorosettes, and was extensively necrotic. It was found in a mediastinal node and involved a partly calcified sympathetic ganglion.

Case 6

R. A. (S.P. 90971), an American, 59 years old, developed nocturia and dysuria which persisted 6 months after prostatectomy elsewhere. It was reported that the prostate showed no tumor. At exploration a pelvic retroperitoneal mass was found. He died 3 days later, and at autopsy the tumor involved both seminal vesicles, the bladder wall, the prostatic bed, and had metastasized to the pelvic and retroperitoneal nodes. Microscopically, the biopsy section showed a tumor composed of solid masses and strands of rather well preserved, rounded, and sometimes slightly elongated hyperchromatic cells which showed no definite differentiation. The autopsy material showed the occasional formation of pseudorosettes and was very suggestive of sympathicoblastoma.

Case 7

D. F. (S.P. 93335), was an American male child, 16 months old. During a routine physical examination hard masses were felt in the umbilical region and the left flank. At exploration there was a retroperitoneal mass which extended from behind the cecum upward, pushing the stomach forward and the transverse colon downward. He was failing rapidly when he left the hospital 18 days later. Sections

taken for biopsy showed a tumor of sympathicoblasts, most of which were necrotic. In a few places pseudorosettes were present.

Case 8

A. A. (S.P. 97260), was an Italian-American female child, 13 months old. Following an attack of pneumonia, she was brought to the hospital with signs of fluid in the chest. When this was not confirmed an exploratory operation was performed and tissue was taken for biopsy from an inoperable mediastinal growth. She received some roentgenotherapy but the tumor did not regress and she was transferred to another hospital at the parents' request. Microscopically, the tumor was made up of sympathicoblasts which occasionally formed pseudorosettes and even immature ganglion cells. A Cajal impregnation showed many delicate neurites among the tumor cells. Occasionally one appeared to originate in a tumor cell.

MATERIAL AND METHODS

The material for explantation was obtained in the course of biopsy of either primary or metastatic sites. Both are equally satisfactory, so long as necrotic areas are avoided. Even the existence of inflammatory tissue associated with necrosis and hemorrhage does not vitiate the results if a fair number of viable tumor cells remain in the specimen used for explantation.

The tissue cultures were handled by the Maximow lying-drop method, modified slightly for our purposes (Murray and Stout, 1942). In the tumor the sympathicoblasts were grouped in solid masses with little supporting framework, thus forming a very friable tissue which broke up readily when cut or otherwise mechanically disturbed. Consequently, in setting out the cultures many fragments of various sizes were scattered through the medium. These small aggregates of pure tumor cells, uncomplicated by the presence of fibrous tissue, have been found to be more satisfactory objects for observation than the main explant.

Diagnosis can be made from the living cultures, but for records and for more detailed study permanent preparations are desirable. The reduced silver method of Bodian (1936), adapted to the cultures as whole mounts, is simple and reliable and furnishes brilliant contrast between neurites and background. For this it is best to fix the cultures in Bouin's fluid for $\frac{1}{2}$ to 1 hour, and ripen in 80 per cent alcohol, with several changes, for at least 2 weeks before treating with silver. The following steps must be controlled with the microscope, but the approximate timing is as follows: protargol solution, 20 to 24 hours; hydroquinone, etc., 5 to 10 minutes; gold chloride, 1 minute; oxalic acid, 1 minute; sodium thiosulfate, $\frac{1}{2}$ minute. Silver impregnation is the only histologic method in our experience which does justice to the finer structures of these sympathicoblasts (see Figures 3 to 5 and 7 to 11), but fairly satisfactory results can be obtained with phospho-

tungstic acid hematoxylin following Zenker fixation and omitting the Mallory bleach.

CHARACTERISTICS OF THE TUMOR IN VITRO

The sympathicoblasts do not migrate to any significant extent, but within 24 hours some of the small round or oval cells cohering in the clumps scattered about the clotted plasma have produced neurites of varying lengths, easily recognizable, and distinct from any form of outgrowth evolved *in vitro* by nonnervous tissues (Fig. 2). These neuroblasts, remaining *in situ,* project filamentous processes which are sometimes beaded, and which grow in the manner of an axone, with pseudopodial ends. The cells are usually monopolar, although sometimes bipolar and very occasionally multipolar. Within 48 hours the neurites have become longer and more numerous, and sometimes have begun to branch (Figs. 1 and 11). In favorable material this branching may become very elaborate as time goes on, so that after a fortnight's cultivation an isolated clump of cells may produce structures resembling a plexus (Figs. 8 and 10).

However, the sympathicoblasts as well as their newly grown neurites are very fragile, and overly responsive to handling; consequently it is best to confine the washing time to a maximum of 5 or 10 minutes, 2 or 3 times per week, and to keep the pH of the washing saline solution below 7.4. The viability of these cells *in vitro* is very variable and is probably connected with the original location of the explant within the tumor, whether it comes from a poorly nourished or moribund area or from a rapidly growing margin.

Necrosis among the cells composing the outer rim of a clump is so common as to be characteristic of this neoplasm *in vitro* (Figs. 4, 6, 8, and 11). This is not at all the case in clumps of cells derived from lymphosarcomas cultivated under the same conditions. Surprisingly, it reverses the pattern of necrosis which is commonly observed in sections of the sympathicoblastoma *in vivo,* in which the best preserved areas tend to lie close to the blood vessels and the pyknotic and necrotic regions are farther from the surfaces at which food, oxygen, and wastes may be exchanged. Such a pattern of necrosis as we observe *in vitro* leads to the inference that our tissue culture medium is not ideal for this material.

The cell body of the sympathicoblast is often about the size of a lymphocyte, though size may vary within a single tumor. The tumor cells may be larger, however, as in our case 7, in which they had two to three times the diameter of a lymphocyte. In this instance there was

considerable variation in size, and some rather large multipolar cells resembling more mature ganglion cells were present. Tumors have been reported (Stout, 1947) which combine ganglioneuromatous areas with others characteristic of the sympathicoblastoma, but we have not obtained one of these for cultivation. The sympathicoblastoma *in vitro* is entirely different from the benign ganglioneuroma, the behavior of which is essentially similar to that of nonneoplastic adult sympathetic ganglion cells (a description of which will be published shortly). These are large multipolar cells, which do not form tissues but remain isolated one from another and are relatively slow to grow and migrate.

Although the production of neurites is the most conspicuous trait of sympathicoblasts *in vitro*, these tumor cells are also prone to adopt epithelial formations. After 4 to 5 days *in vitro*, tongues or cords of cells appear, usually ending in a filamentous process (Figs 6 and 8). Cell boundaries in such an outgrowth are often indistinct, giving the whole mass the appearance of a syncytium. Nuclei are sometimes lobate or kidney-shaped, and since mitotic figures are only very rarely observed among them *in vitro* it is assumed that these nuclei may multiply by direct division. Within a week or more, flat membranes may be seen in favorable cultures; these differ from typical epithelial membranes in that they frequently develop dendritic outgrowths (Fig. 7). The sympathicoblastoma thus appears to be related to the neuro-epithelioma, one example of which we have been able to study in tissue culture (Stout and Murray, 1942). This exceedingly undifferentiated tumor, arising in the radial nerve, produced membranous sheets of epithelium when cultivated, but no neurites. The neuro-epithelioma might possibly be compared to the neural plate stage in nervous development. It seems probable that the sympathicoblastoma typifies a state of differentiation similar to that found in the neural tube stage of the embryo, since the tumor cells are small, capable of forming membranes *in vitro* (as does columnar epithelium from other germ layers), and are unaccompanied by satellite cells of any description. The rosette formations found in sections of neuroblastomas in general have been thought to represent cross sections of neural tubes formed in these neoplasms.

By the fourth day *in vitro*, fibroblasts are fairly numerous. At the end of 1 week, neurites and other evidences of the sympathicoblasts in the explant are usually obscured by the fibroblastic growth, but the islands of pure neuroblasts scattered throughout the clot may remain distinct for at least 1 month (Fig. 5), the duration of our observations.

Explants of these tumors frequently contain considerable numbers

of lymphocytes and other blood elements, which migrate out into the clot. Differences in form are usually sufficient to distinguish these from the neoplastic cells, but the distinction may be heightened by vital staining with basic dyes. The living sympathicoblasts stain quickly with neutral red applied supravitally in a dilution of 1:10,000. The stain may appear in the cell as a compact group of small granules occupying a juxtanuclear position, or as a few scattered granules in a diffusely pink cytoplasm. In either case the sympathicoblast can be distinguished readily from the normal or neoplastic lymphocyte, which, although it may aggregate in clumps, does not take up neutral red at all. The addition of a dilute solution of Janus green B to the neutral red solution serves to emphasize the distinction further, since the sympathicoblasts contain few or no Janus green staining particles, while the lymphocytes take up the dye readily.

DISCUSSION AND SUMMARY

The cultivation of the sympathicoblastoma *in vitro* provides a more rapid as well as a more certain means of *identifying this tumor* than the customary histologic section methods. Frozen sections are often unsatisfactory; they are particularly unreliable in distinguishing the sympathicoblastoma from members of the lymphosarcoma group or from the small-celled carcinomas.

Small fragments of the tumor, isolated in the medium of clotted plasma, can be counted on to produce neurites within 24 hours. This faculty, coupled with the tendency to marginal necrosis and the affinity of the tumor cells for neutral red applied supravitally, provides a very satisfactory means of differential diagnosis between this tumor of nervous origin and the lymphosarcomas and Ewing's tumor with which it is sometimes confused clinically. As cultivation is continued, from 2 to 5 days, the whole picture becomes increasingly clear and distinctive. There is great advantage in being able to see the whole cell with all its processes and extensions, as well as the characteristic patterns in which the cells group themselves. When this can be done, the sympathicoblasts present a very different appearance from lymphocytes or lymphoblasts or from small carcinoma cells.

Of the eight tumors which we have studied *in vitro*, two have occurred in adults, aged 33 (case 4) and 59 (case 6) years, respectively. Because of their equivocal histologic features these could not be accepted as unquestionable sympathicoblastomas without the examination *in vitro*.

BIBLIOGRAPHY

Bodian, D. A new method for staining nerve fibers and nerve endings in mounted paraffin sections. *Anat. Rec.*, 1936, 65, 89–97.

Murray, M. R., and Stout, A. P. Demonstration of the formation of reticulin by Schwannian tumor cells *in vitro*. *Am. J. Path.*, 1942, 18, 585–593.

Stout, A. P. Ganglioneuroma of the sympathetic nervous system. *Surg., Gynec. and Obst.*, 1947, 84, 101–110.

Stout, A. P., and Murray, M. R. Neuroepithelioma of the radial nerve, with a study of its behaviour *in vitro*. *Rev. canad. de biol.*, 1942, 1, 651–659.

_____

[*Illustrations follow*]

DESCRIPTION OF PLATES

PLATE 72

FIG. 1. Case 8. Living isolated clump of sympathicoblasts, with neurites (N) having pseudopodial ends; 48 hours *in vitro.* × 290.

FIG. 2. Case 8. Living clump of sympathicoblasts with neurites (N); 24 hours *in vitro.* × 290.

FIG. 3. Case 4. Small clump of sympathicoblasts (S) with neurites (N); F indicates a fibroblast; 10 days *in vitro.* Bodian method. × 380.

FIG. 4. Case 4. Clump of sympathicoblasts (S) with branching neurite (N), and marginal necrotic area (Ne). Flattened fibroblasts (F) are shown for comparison of size; 10 days *in vitro.* Bodian method. × 380.

FIG. 5. Case 5. Isolated clump of sympathicoblasts (S) with neurites (N) surviving 30 days. F is a fibroblast. Bodian method. × 255.

Murray and Stout

Sympathicoblastoma Cultivated *in Vitro*
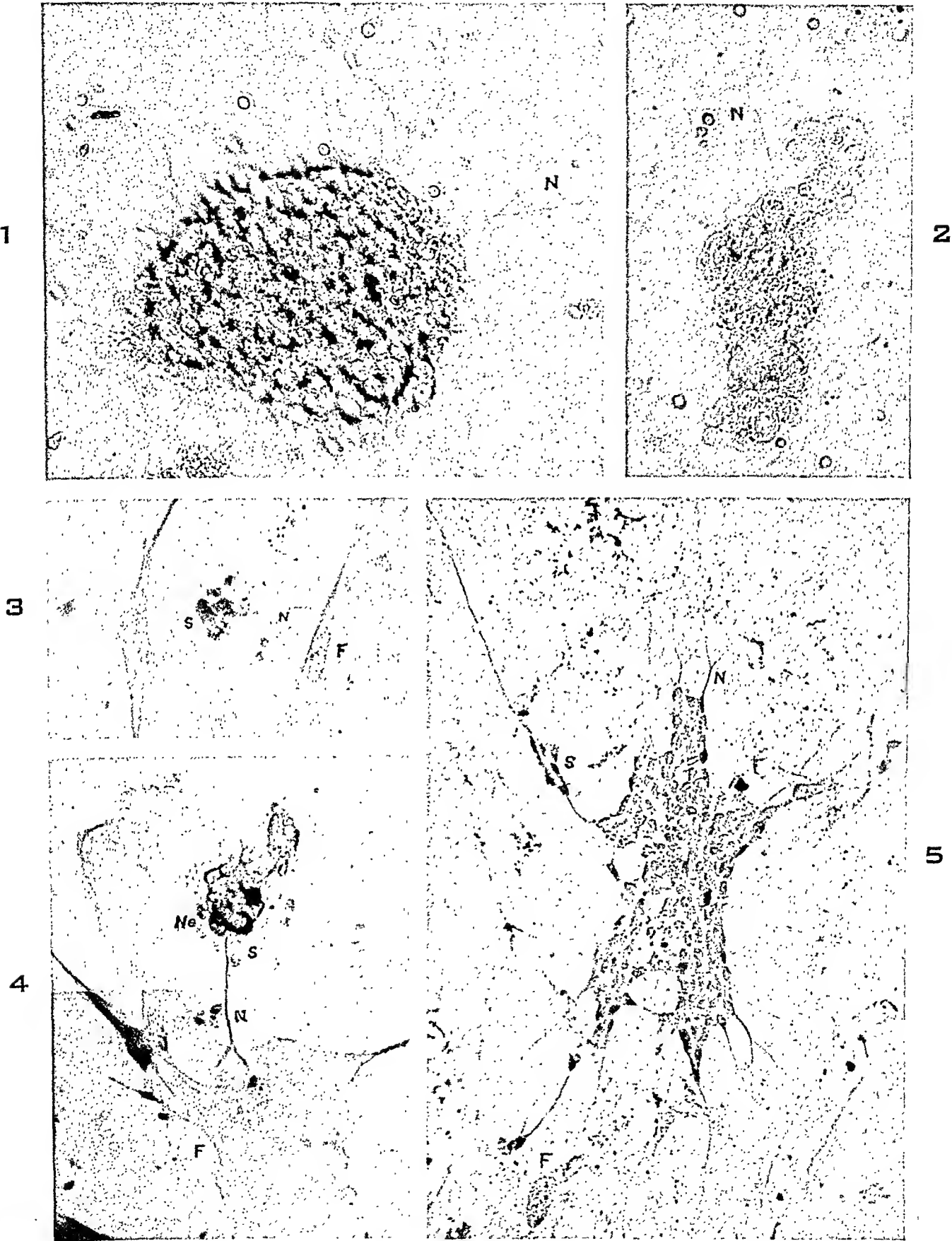
PLATE 73

FIG. 6. Case 3. Isolated clump with projecting tongues of neuro-epithelium (N-E) ending in neurites (N), and marginal necrosis (Ne); 5 days *in vitro*. Phosphotungstic acid-hematoxylin stain. × 305.

FIG. 7. Case 3. Sympathicoblasts growing as a flat epithelium (N-E) with neurites (N). Fibroblasts (F). 17 days *in vitro*. Bodian method. × 245.

FIG. 8. Case 3. Clump of sympathicoblasts with pyknotic center, necrotic margin (Ne), epithelial tongues (N-E), and many branched and beaded neurites (N); 5 days *in vitro*. Bodian method. × 220.

FIG. 9. Case 5. Membranous clump of sympathicoblasts (S) with very long, beaded neurites (N); 7 days *in vitro*. Bodian method. × 245.

FIG. 10. Case 3. Neurites (N) tending toward plexus formation; necrotic material (N-E); young fibroblasts (F); 17 days *in vitro*. Bodian method. × 140.

FIG. 11. Case 6. Clumps of sympathicoblasts (S), with necrotic margins (Ne) and neurites (N) at 48 hours. Bodian method. × 360.

Plate 74
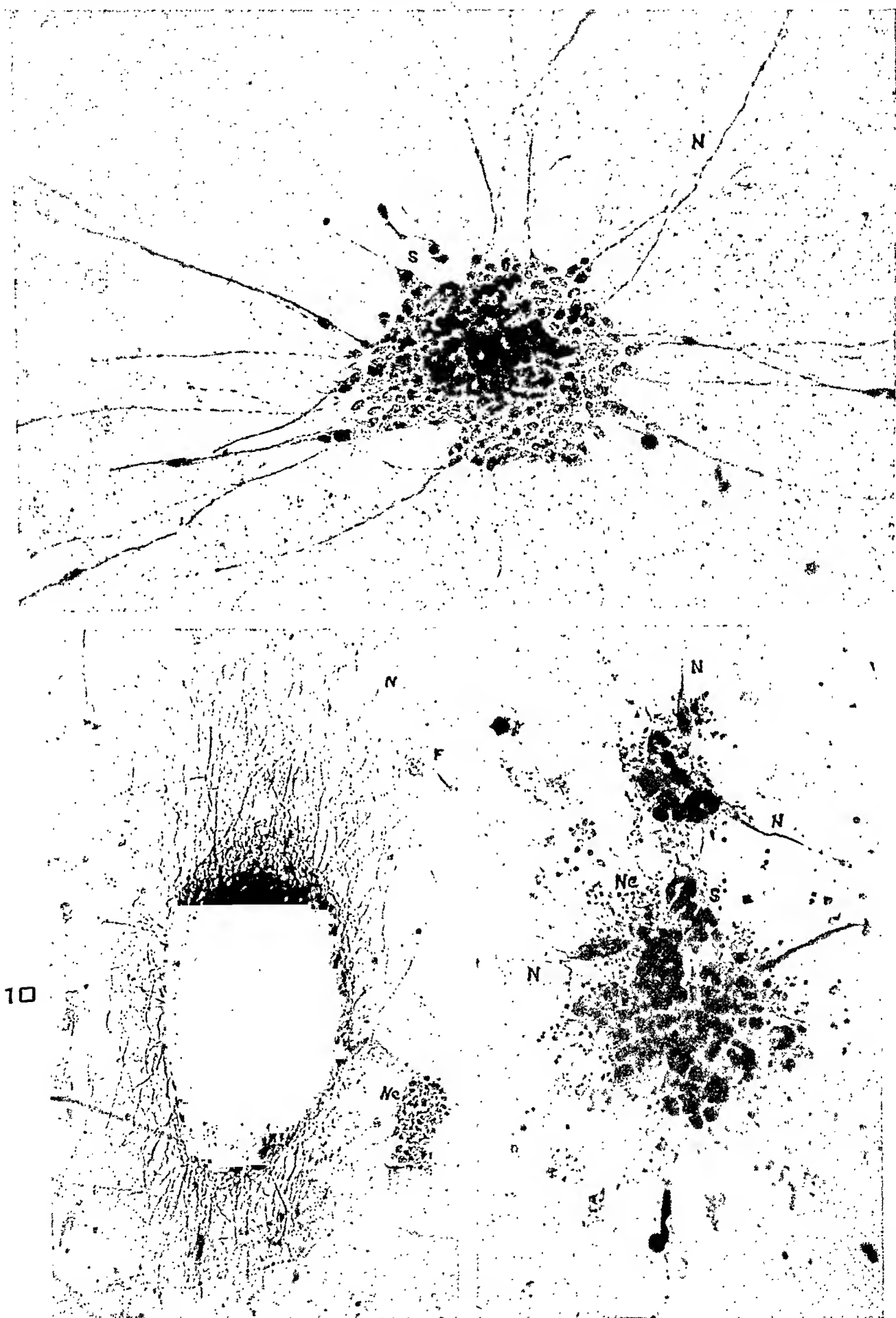
INTESTINAL LIPODYSTROPHY OF WHIPPLE
REPORT OF A CASE AND ANALYSIS OF THE LITERATURE *

MAY SHERMAN ROSEN, M.D., and SAMUEL H. ROSEN, M.D.

(*From the Laboratory Division, Montefiore Hospital, New York 67, N.Y.*)

In 1907 Whipple [1] described a disease which he believed to be unique in the literature and for which he proposed the name intestinal lipodystrophy. It was characterized clinically by steatorrhea, progressive weakness, and weight loss; and pathologically by dilatation of the lacteals in the small intestine, of the mesenteric lymphatics, and of the sinuses of the mesenteric lymph nodes, with accumulation of intracellular and extracellular fatty materials at these sites.

Apparently no similar case was reported until 1923, when Blumgart [2] described 3 fatal instances of what he called malabsorption of fat, which he felt resembled Whipple's case in many respects. Unfortunately, Blumgart's cases are difficult to evaluate, due to apparent inconsistencies between his abstracts of the autopsy protocols and his summary of them. Later authors have sometimes accepted all 3 of Blumgart's cases and sometimes only his second. Reports of unexplained diarrhea or steatorrhea with pathologic findings reminiscent of Whipple's original description have become increasingly prominent during the past 10 years. Because it is not yet clear whether Whipple's disease is a distinct entity, and if it is, exactly what features constitute the entity, there has been much confusion as to which cases should be included under the designation intestinal lipodystrophy. Some cases have been called Whipple's disease by some authors and discarded or overlooked by others. At least one or two other cases, not included in recent reviews, probably belong in the group. Possibly there are many others, lost in the literature under various designations, which could be included.

In 1936 Jarcho [3] presented what he believed to be the third case of Whipple's disease, since he accepted only Blumgart's [2] second case. The following year Hill [4] described a case of "mesenteric chyladenectasis," which was in many respects similar to Whipple's original description, but which Hill himself did not identify with this group. Reinhart and Wilson,[5] in 1939, reported a case of intestinal lipodystrophy and reviewed the descriptions of Whipple,[1] Blumgart,[2] Jarcho,[3] and Boeck.[6] Boeck's case had been presented briefly in 1938, in a discussion of pancreatic insufficiency, as an instance of steatorrhea.

The next review of the disease as such was by Sailer and McGann,[7]

in 1942. These authors, while reporting an additional case, reviewed the literature and included in the group the cases of Whipple, Blumgart (all 3), Jarcho, Hill, Boeck, and Reinhart and Wilson. In addition, they added those of Fleischmann [8] (1930), and Korsch [9] (1938), previously overlooked. Thus, with their own case, they brought the number up to 11. In the same year (1942) Pearse [10] presented a case diagnosed during life, the patient still being alive at the time of publication. Whipple himself had reviewed the slides of the biopsy material and believed the cellular picture to be similar to that in his own case. However, the intestine and mesenteric lymph nodes were not examined. If Pearse's description is to be considered consistent with Whipple's disease, then the second case of Collins and Berdez [11] certainly should be included.

From this period the literature became more and more inconsistent. Apperly and Copley,[12] in 1943, reported what they considered to be the 12th case. Actually, if all the above-mentioned cases were included, it would be the 14th since these authors made no mention of the cases of Pearse[10] or of Collins and Berdez.[11] In their report they included a tabulation of the symptoms and pathologic findings in the 12 cases.

In November, 1945, 2 case reports of Whipple's disease appeared in different journals. One was that of Amsterdam and Grayzel,[13] who reproduced the tabulation of Apperly and Copley,[12] and added to it their own case and that of Vaux [14] (1943). However, it is probable that they should have included also a case to which Vaux had referred, that of Glynn and Rosenheim [15] (1938), which Vaux considered to be in a class with her own case and that of Hill.[4] The other case was that of Fitzgerald and Kinney,[16] who considered theirs to be the 8th instance of Whipple's disease. Actually, if all the others are included, except that of Amsterdam and Grayzel which appeared simultaneously with it, this must be considered the 17th case. Fitzgerald and Kinney included in the group the following cases: Whipple, Blumgart (second case only), Jarcho, Reinhart and Wilson, Sailer and McGann, Korsch, Pearse, and their own. They excluded Blumgart's first and third cases, and those of Fleischmann, Boeck, and Hill. They apparently were not aware of the reports of Apperly and Copley, Vaux, Glynn and Rosenheim, and Collins and Berdez.

Finally, attention should be called to Thannhauser's [17] discussion of Whipple's disease. Thannhauser believed that the descriptions of Whipple [1] and of Reinhart and Wilson [5] coincided with those in cases of "xanthomatous transformation" of the mesentery, in which accumulations of foamy cells in the mesenteric fat are incidental findings at autopsy and are probably secondary to inflammation of the fat or to fat

necrosis from various causes. We shall describe such foci in our own case. Unfortunately, we have not been able to find any mention of such lesions in either Whipple's or Reinhart's descriptions; and the photomicrograph from Reinhart's case which Thannhauser reproduced was apparently obtained through a personal communication. Thannhauser believed that the cases of Jarcho [3] and Blumgart [2] do not belong here, as those authors did not describe such xanthomatous transformation. Needless to say, these comparatively insignificant xanthomatous deposits in the mesentery might easily have been overlooked by Jarcho and Blumgart, as in the other cases, in view of the more striking pathologic findings. Certainly it would seem that the cases referred to by Thannhauser have little in common with our own case or the other reported cases of intestinal lipodystrophy; and that in our own case, at least, the mesenteric accumulations of foam cells were secondary to the intestinal and lymphnodal deposits, perhaps from a spilling over of fats from ruptured lymphatics, rather than representing the primary lesion.

It is our opinion that the typical lesions of Whipple's disease as described by him were the massive accumulations of intracellular and extracellular fat in the small intestine and its draining lymph nodes, with dilatation (probably resultant) of lacteals and mesenteric lymphatics; and that if these are considered the pathologic criteria for the disease, the only cases that definitely can be included after Whipple's case, at least until more is known about the pathogenesis, are, in chronologic order, those of Jarcho, Hill, Korsch, Glynn and Rosenheim, Reinhart and Wilson, Sailer and McGann, Apperly and Copley, Vaux, Amsterdam and Grayzel, and Fitzgerald and Kinney. We would exclude those of Blumgart because of the incompleteness and inconsistencies of his descriptions; of Pearse, because neither the intestine nor mesenteric lymph nodes were examined; and of Fleischmann, because the intestine apparently was not involved. Boeck's case is doubtful, because the involvement of mesenteric nodes, found at laparotomy, was no longer present at autopsy 4 months later, although fat deposits were noted in the wall of the small intestine. The case of Collins and Berdez is also doubtful because of inadequate description of the intestinal lesions. It is possible that when more is known of the pathogenesis of this disease, at least some of the cases which we have excluded may be considered early or less advanced instances of the disease.

The following case satisfies the criteria we have suggested above, and we therefore consider it to be the 12th such case. If all the cases reviewed above should be included, however, our case brings the total, so far as we have been able to ascertain, to 19.

REPORT OF CASE

The patient, I. W. (autopsy no. 10205), was a Russian-born white female, 54 years old, who was seen on April 10, 1945, by a private physician. She complained of increasing abdominal pain, weight loss of 10 lbs. in 6 months, poor appetite, and obstinate constipation.

On April 22, 1945, the patient entered Bronx Hospital. Her complaints then were weight loss of 15 lbs., weakness, and diarrhea of 2 weeks' duration. She stated that she had 4 to 5 bowel movements daily and that the stool was dark green and showed no blood. Prior to the onset of diarrhea, she had been constipated for several years, taking senna without effect.

On examination, the patient was lying in bed, in no apparent distress. Malar telangiectases were noted. The blood pressure was 112/60 mm. Hg. Heart and lungs were negative. The abdomen was large and distended. A mass, later interpreted as spleen, was felt 4 fingersbreadth below the costal margin in the left side. There was no peripheral edema. Reflexes were normal.

Laboratory Findings. The urine showed a trace of albumin. Examinations of the blood on two occasions revealed the hemoglobin to be 72 and 68 per cent; red blood cells, 4.61 and 4.27 million; white blood cells, 14,500 with 37 per cent polymorphonuclear leukocytes, 54 per cent lymphocytes, 8 per cent monocytes; and white blood cells, 11,700 with 43 per cent polymorphonuclear leukocytes, 54 per cent lymphocytes, 1 Türk cell. Icterus index was 7. Total cholesterol was 142.9 mg. with 57 per cent esters. Stool culture was negative for the typhoid-dysentery group, and there was no occult blood. Sternal puncture revealed normal bone marrow. Total proteins were 6.33 gm., with 3.32 gm. of albumin, and 3.01 gm. of globulin. Takata-Ara test was negative. Cephalin flocculation test was 2 plus. The Wassermann test was doubtful and the Kahn test negative on one occasion, but later each was reported as 4 plus. Roentgenograms of the abdomen showed the spleen to be enlarged to the umbilicus, and a stone of biliary type in the gallbladder region.

The patient was afebrile throughout her stay. On April 26 a moderate amount of fluid was noted in the abdomen. She was discharged on May 15, 1945, with the final diagnosis of cirrhosis of the liver and lues.

She entered Montefiore Hospital on May 29, 1945. She then complained of having had "heart disease" for 3 months, diarrhea and weight loss for 2 months. There had been no vomiting and no tarry or bloody stools. She claimed to be having eight bowel movements a day. There was also a dubious history of numerous bouts of congestive circulatory insufficiency.

Examination revealed a "noisy" female with no dyspnea or orthopnea. Temperature was 98°F.; pulse, 90; blood pressure, 108/62 mm. Hg. Pupils were contracted and reacted poorly to light. There was an apical systolic murmur and an accentuated second aortic sound. The abdomen was distended. The spleen was felt 4 fingersbreadth below the costal margin. There was 1 plus pretibial edema. Reflexes were hypoactive. The impressions were: Banti's syndrome; general paresis; acute gastro-enteritis.

Laboratory Findings. The Wassermann and Kahn tests were 4 plus; Kline test, 3 plus. Urine: specific gravity, 1.020 and 1.013, with albumin 0 to a trace; glucose, a trace to 2 plus; up to 10 white blood cells per high power field. No blood count was done.

The patient received antiluetic therapy in the form of bismuth in oil, liver injections, and mercupurin. The diarrhea subsided somewhat. On July 3, 1945, abdominal paracentesis released 2500 cc. of straw-colored fluid with 0.8 per cent protein. During the last few weeks of her life increasing "muddy" pigmentation of the skin was noted. She had periods of confusion, hysteria, and depression. Her mental status was considered to be due to tertiary lues.

On July 17, 1945, the patient suddenly had copious coffee-ground vomitus, went into shock, and appeared to aspirate much vomitus, soon becoming livid. All measures to relieve her were futile and she died about 4 hours after the onset of these symptoms.

AUTOPSY FINDINGS

Autopsy was performed 2 hours post-mortem by Dr. Ruth Lubliner and was reviewed by us. The body was that of a fairly well developed and nourished middle-aged white female, 57 inches in length. There was cyanosis of face, lips, ears, and neck. Numerous scratches were noted on the breasts, abdomen, and arms, and petechial and larger hemorrhages were present over the chest. There were brownish crusts in the nostrils and on the lips. Moderate dependent edema was present. Peripheral lymph nodes were not enlarged. The abdomen was distended and the abdominal cavity contained about 2000 cc. of opalescent, milky, somewhat yellowish fluid, in which a large amount of soft, opaque, whitish, curd-like material was suspended. The serosal surface of the small intestine and the pelvic peritoneum were covered with shreds and sheets of similar material. There were a few easily torn fibrous adhesions. A fairly large amount of fat was present in the usual depots. The liver extended about 6 cm. below the costal margin. The spleen was enlarged, displaced anteriorly, and extended along the costal margin from the mid-axillary line to the mid-clavicular line. There was marked engorgement of all superficial and deep veins. The pleural cavities were free of fluid. Some fibrous adhesions were present over the right lung.

There were submucosal hemorrhages in the larynx, trachea, and pharynx; and subpleural punctate hemorrhages over the lower lobe of the right lung. The lungs showed marked edema. The bronchi contained no blood or aspirated material. There was considerable variation in the appearance of the hilar and tracheobronchial lymph nodes. Many, as commonly seen in this location, were small, fairly firm, and deeply anthracotic. However, several of the bronchial nodes, on the left side only, and especially those just below the bifurcation of the trachea, were enlarged to about 1.5 or 2 cm. Some of these were fluctuant and opaque grayish white to yellowish. On section they were found to be largely reduced to a shell enclosing a thick opaque fluid material with a somewhat greenish tint probably due to admixture with anthracotic pigment. Others were moderately firm, partly deeply anthracotic, and partly grayish white with numerous soft, opaque whitish or yellowish white foci of 1 mm. or less and very little anthracotic pigment. The heart weighed 240 gm. and was flabby. The aorta and coronary arteries showed moderate arteriosclerosis. The liver

weighed 1800 gm.; section revealed swollen reddish to yellowish brown lobules. The gallbladder contained 40 cc. of viscid bile and several irregular, friable, dark green calculi. The spleen weighed 380 gm. and was flabby. The capsule was irregularly thickened. The cut surface was mottled reddish gray with small, dark red foci, and did not scrape. The pancreas appeared normal. The right adrenal weighed 7.5 gm. and the left, 8 gm. The cortex was broad and yellowish brown. The kidneys each weighed 150 gm. and were flabby. The capsule stripped easily from a smooth surface. Bone marrow of the lumbar vertebrae was pale red-brown. The thyroid gland weighed 12 gm.; translucency was slightly decreased. Examination of the brain and cord was not permitted.

The outstanding changes were in the gastrointestinal tract and abdominal lymph nodes. The lower third of the esophagus showed a granular hemorrhagic mucosa. There was marked spasm of cardia and pylorus. The stomach was contracted and contained a small amount of brownish mucus.

The small intestine was distended with a large amount of pale yellowish, very soft, unformed material. The wall was thickened and rather rigid, the lumen dilated up to 1½ times the normal circumference. The valvulae conniventes measured up to 2 to 3 mm. in thickness and up to 6 mm. in height, and between them the wall measured up to 1.5 cm. in thickness. The entire mucosal surface had an opaque, whitish or yellowish white, rather milky appearance, which on the whole was diffuse, but which here and there left patches of normal color with scattered opaque, yellowish white spots (Fig. 1-b). On closer inspection the coloration was seen to be due to myriads of opaque, yellowish white, slightly elevated foci, pinpoint to pinhead in diameter, and up to 0.5 mm. or more in height, closely spaced and giving the surface a granular or plush-like appearance. These foci, apparently enlarged villi, became confluent in large areas, especially over the circular folds. Cross section of the intestinal wall revealed occasional opaque, whitish or yellowish white spots, probably dilated lymphatics, in submucosa, muscularis, and serosa (Fig. 1-a). Similar spots and streaks could be seen on the serosal surface. Occasionally there were soft nodular elevations of this color up to about 1 cm. in diameter and 4 or 5 mm. in height. On incision milky fluid was released from these, leaving a smooth-lined space, presumably a dilated lymphatic. Similar milky fluid seemed to exude from the entire cut surface of the intestine. These foci in the serosa seemed to be most numerous along the mesenteric attachment, where they could frequently be seen continuing into the mesenteric fat. The changes described above began abruptly

immediately beyond the pyloric ring and ended with the same abruptness at the ileocecal valve; they were somewhat more pronounced in the duodenum and jejunum than in the ileum. The Peyer's patches and solitary lymph follicles were not unusual. The appendix was normal. The entire colon was filled with fecal material similar to that in the small intestine. There were no formed contents.

Striking changes were seen in all abdominal lymph nodes. They ranged from 0.5 to 2 cm. in greatest dimension, the larger being mesenteric and periesophageal. The nodes were discrete, soft, and sometimes fluctuant. On section the smaller ones consisted partly or entirely of soft, friable, opaque, whitish, cheesy material. The larger nodes were reduced in part or completely to cyst-like structures containing a milky fluid or opaque, yellowish, creamy material.

There was marked thickening of the mesentery, which, in addition to the altered lymph nodes, contained numerous opaque, whitish or yellowish white pinhead and larger spots and streaks, seen through the peritoneum and on the cut surface (Fig. 1-a). On section these released a milky fluid; they were interpreted as dilated lymphatics. There were also many irregular, opaque, pale yellowish areas from pinhead to a few cm. in size in the mesenteric fat (xanthomatous foci). The omentum showed similar changes. In its upper portion there was a large cyst-like space about the size of a plum. It was lined by a thick layer of soft, friable, whitish, curd-like material, and was interpreted as an encapsulated chylous effusion.

Microscopic Examination

Examination of the tissues other than small intestine, lymph nodes, and mesentery confirmed the gross findings.

Small Intestine. There was marked enlargement of most of the villi of the small intestine, both in length and in breadth, producing bizarre club-shaped, pear-shaped, or globoid forms (Fig. 2). Expansion was due mainly to the accumulation of large amounts of deep pink coagulum which appeared to lie mainly in the stroma and was thinly scattered with lymphocytes and a few macrophages. In places this material still occupied large spaces, lined by endothelial cells, presumably dilated lacteals. There was an excess of lymphocytes, plasma cells, and eosinophils throughout the propria, and some proliferation of fibroblasts and young capillaries. The submucosa contained a few dilated lymphatics filled with a paler pink coagulum, sometimes with a few large foam cells; or occasionally distended with a mass of foam cells. Between these the submucosa appeared edematous and contained a thin scattering of lymphocytes, plasma cells, and macrophages,

as well as masses of foam cells. In only one or two places a large space, apparently representing dissolved-out lipoid, was surrounded by elongated multinucleated cells which had curved around it, and beyond these a zone of lymphocytes and macrophages. Greatly dilated lymphatics lay in the subserosa; these contained pale granular, highly vacuolated or foamy pink-staining material. In places in the subserosa there was also considerable deeper pink coagulum, containing a few foam cells, lying free. Here and there a few small masses of fibrin were present in the serosa. The latter was considerably thickened by proliferating fibroblasts and capillaries and by considerable diffuse and more marked focal infiltration of lymphocytes, a few plasma cells, and numerous macrophages. The macrophages were often greatly swollen and foamy and were more diffusely scattered than the other infiltrating cells. The foamy cells also appeared in well defined clumps in the subserous fat.

Lymph Nodes. Sections of several of the abdominal nodes, including mesenteric, periesophageal, and peripancreatic, and of a left tracheobronchial node showed striking changes (Figs. 4 and 5). The lymph sinuses, both cortical and medullary, were widely dilated and distended with deep pink-staining material, sometimes homogeneous, sometimes finely or more coarsely vacuolated, and so dense that often it had fragmented under the knife like colloid. The intervening lymphatic cords were markedly compressed and reduced to rather narrow strands densely packed with lymphocytes, and in places infiltrated with plasma cells and occasional eosinophils. Mingled with the coagulum were large numbers of tremendously swollen foamy macrophages, together with a sprinkling of lymphocytes, plasma cells, and, in places, numerous polymorphonuclear leukocytes. Where there was less coagulum, the lipophages lay in closely packed masses and sheets. Scattered foam cells also were present throughout the lymphatic cords. In some of the nodes there were a few small hemorrhages into the sinuses and small accumulations of free and phagocytosed blood pigment. In a periaortic node, the pink-staining coagulum was not found, but there was a scattering of foamy cells through it. In this node there also were proliferation and desquamation of reticulo-endothelial cells into dilated sinuses, and many plasma cells, polymorphonuclear leukocytes, and especially eosinophils in the sinuses and infiltrating the lymph cords.

Mesentery. In addition to the changes in the lymph nodes already described, the mesenteric lymph vessels were widely dilated and filled with the same material as the nodal sinuses (Fig. 3). Blood vessels were engorged. The fat showed slight diffuse and more marked focal infiltration of lymphocytes and plasma cells. Scattered throughout the

fat were small and large accumulations of foam cells, reaching xantho-matous proportions. These foam cells, like those encountered previ-ously, varied from slightly larger than the usual macrophages to per-haps 30 or 35 μ. They had a sharply defined, sometimes thick, deep pink cell membrane and a tremendously ballooned-out, frothy, fairly uniformly vacuolated, pale pink cytoplasm. The nucleus was usually eccentric, small, dark, round, and often pyknotic.

Special stains were done on sections of a mesenteric lymph node and small intestine. With sudan IV the coagulum in the lymph vessels, lacteals and in the interstitial tissue of the bulbous villi as well as the cytoplasm in the foam cells took a brilliant reddish orange hue, varying

TABLE 1

Results of Chemical Analysis

	Mg. per 100 mg. of dried tissue	
	Mesenteric lymph nodes	Jejunum
Total lipids	73.5	53.4
Phosphatids (26 × lipid P)	5.8	4.9
Cholesterol	6.1	4.2
Total fatty acids	64.0	41.5
Free fatty acids	12.1	5.7
Saponification number	166	150

somewhat in intensity. With Nile blue sulfate there was considerable variation from the bright pink of the neutral fat, seen in the normal mesenteric fat and probably the predominant hue in the fatty deposits, through pinkish lavender and bluish lavender to deep royal blue.

Chemical analysis of an alcohol-ether extract of formalin-fixed mesenteric lymph nodes and a section of jejunum was done by Dr. Emil J. Baumann. Several heavily involved mesenteric lymph nodes and a few small segments of jejunum were freed as completely as pos-sible of their external adipose tissue and pooled for the chemical analy-sis. This analysis showed a high lipid content, consisting predomi-nantly of neutral fats, with lesser amounts of phospholipids, choles-terol, and free fatty acids. The saponification number was lower than that for normal tissue (Table I).

Anatomic Diagnoses. Latent lues (clinical); intestinal lipodystrophy (Whipple's disease) involving small intestine, mesentery, and omen-tum, and mesenteric, peripancreatic, periportal, perigastric, peri-esophageal, periaortic, and left tracheobronchial lymph nodes; chylous ascites; distention of small intestine with fecal material; petechial and larger hemorrhages in skin, larynx, trachea, pharynx, esophagus, pleura, and vaginal mucosa; engorgement of all peripheral and deep

veins; edema of lower extremities and dependent part of abdomen; marked cyanosis; chronic splenitis with splenomegaly; peritoneal adhesions; subacute esophagitis; edema of lungs; severe parenchymatous degeneration of heart, liver, and kidneys; generalized arteriosclerosis; melanosis coli; multiple lipomas of cecum and ascending colon; cholelithiasis; large adenoma of right kidney; patchy fibrosis of upper lobe of right lung; pleural adhesions; small cholangioma of liver; small intramural uterine fibromyomas; small fibromyoma of stomach.

COMMENT

As in the previously reported cases of Whipple's disease, our patient was of middle age, and had gastrointestinal symptoms of relatively short duration, complaining of abdominal discomfort, asthenia and weight loss, and diarrhea. As in many or all of the other cases, there were pigmentation of the skin, peripheral edema, moderate anemia, low blood pressure, and absence of fever. Pathologically, the outstanding findings were limited to the small intestine, regional and some distant lymph nodes, and the mesentery, and were unlike those described for any other known disease.

Unlike any of the other reported cases, except Blumgart's[2] second, and that of Collins and Berdez,[11] both of which we consider to be questionable examples of this disease, our patient was a female. There was no history of arthritis, such as has been described in several cases, and clinically, at least, no definite steatorrhea. In addition, our case presented a few interesting features, noted in at least some previous reports, which may or may not be a part of the syndrome. For one thing, the sudden death, clinically considered asphyxial in nature, could not be explained adequately at autopsy. Several other patients died suddenly, without satisfactory explanation. Whipple's[1] patient died in respiratory distress 2 days following surgical exploration of the abdomen. In Reinhart and Wilson's[5] case, the patient suddenly became cyanotic and died. Fitzgerald and Kinney's[16] patient died suddenly at home 24 hours after leaving the hospital. Sailer and McGann's[7] patient also died suddenly following an attack of acute abdominal pain. Blumgart's[2] first patient and Boeck's[6] patient both died apparently in acute cardiac decompensation. In none of these cases was death satisfactorily accounted for on the basis of the anatomic findings.

Moderate hepatosplenomegaly was noted in several of the reports. In Reinhart and Wilson's[5] case it must be discounted, since there was portal cirrhosis. In Fitzgerald and Kinney's[16] case the clinical picture and the findings in the surgically removed spleen were consistent with

a diagnosis of hemolytic jaundice. Whipple[1] gave the weights of liver and spleen as 2570 and 375 gm., respectively, the latter showing "chronic splenitis." Korsch[9] merely noted that the liver and spleen were enlarged. In Apperly and Copley's[12] case the liver weighed 2200, the spleen, 320 gm.; microscopically, the former presented peculiar granulomatous nodules; the latter, some nonspecific changes. In our own case the liver was perhaps slightly enlarged (1800 gm.), the spleen moderately (380 gm.), apparently due to a combination of congestion and splenitis.

Leukocytosis with relative and absolute lymphocytosis was described by Reinhart and Wilson,[5] in whose case it led to a clinical diagnosis of benign pseudoleukemic lymphocytosis; by Fitzgerald and Kinney,[16] whose case was followed for some time as lymphatic leukemia and the patient treated with radioactive phosphorus; and was present in our own patient, in whom the diagnosis of lymphatic leukemia was also entertained for a period, especially in view of the accompanying splenomegaly. Fitzgerald and Kinney were unable to give any explanation for the lymphocytosis other than that it was an "unusual type of lymphocytic or histiocytic response of unknown causation." It is our belief, however, that the lymphocytosis may be related to the lymph stasis in the small intestine. Bunting and Huston[18] showed that the number of lymphocytes entering the blood stream daily from the thoracic duct is greater than the total number demonstrable in the blood at any one time, and postulated that lymphocytes in large numbers are excreted from the blood stream into the lumen of the alimentary tract. According to Thompson,[19] streams of lymphocytes flow from the lymphoid tissue of the alimentary canal in two directions: towards the lumen of the bowel and towards the subserous lymphatics. In the intestine, the migration of lymphocytes is predominantly into the lumen of the gut.

On the other hand, Erf[20] demonstrated in rabbits that the rôle of the gastrointestinal tract in the excretion of lymphocytes is readily taken over by other forces within the body. We, ourselves, have seen a leukocytosis of 160,000 (100 per cent polymorphonuclear) develop after abdominoperineal resection in a patient with chronic ulcerative colitis, drop to 40,000 in 1 day, and return to normal levels within 2 weeks, accompanied throughout by a normal bone marrow. The interpretation * was that the diseased colon had called forth a marked local increase in polymorphonuclear leukocytes; and that with its sudden removal these cells, still being produced in excess by the marrow and no longer being excreted at the usual site, had piled up in the peripheral blood. Un-

* Dr. Samuel Melamed, Montefiore Hospital.

doubtedly the balance between production and destruction of leuko-
cytes is a delicate one; and when a portion of the mechanism is put out
of commission, the ability of the rest of the system to take over either
or both functions will vary with the individual. It is conceivable that
the accumulation of fat in the intestinal mucosa in Whipple's disease
can impede the usual excretion of lymphocytes through this mucosa;
and that in the 3 instances of lymphocytosis enumerated above, the
other excretory sites had not completely substituted for the bowel.
Also to be taken into consideration is the rôle that leukocytes play in
the transport of fats,[21] with the possibility that the massive deposits
of fat in the intestine may be a stimulus to mobilization of lymphocytes.

Morphologically, our case demonstrated another feature of interest
in the extent of involvement of the lymph nodes. In most of the previ-
ous reports, only the mesenteric nodes, sometimes with the peripan-
creatic, showed the characteristic changes. In a few, the retroperitoneal
nodes were also involved. Fitzgerald and Kinney[16] found also slight
involvement of paratracheal nodes, and Reinhart and Wilson,[5] and
Apperly and Copley,[12] of mediastinal nodes. Korsch[9] described, in
addition to mesenteric and retroperitoneal nodes, enlarged mediastinal,
paratracheal, and cervical nodes presenting the same picture. In our
case all of the abdominal nodes were involved, including the peri-
esophageal and periaortic. In addition, the tracheobronchial nodes, on
the left side only, showed typical changes, thus nicely demonstrating
the drainage of the left bronchomediastinal trunk into the thoracic
duct. The right bronchomediastinal trunk normally empties into the
right lymphatic trunk or junction of right internal jugular and sub-
clavian veins. The variation in the extent of lymph node involvement is
not difficult to explain. When back pressure reaches a certain level in
the receptaculum chyli, it will be communicated to the lumbar trunks
and so to the retroperitoneal nodes. With the mounting tide of pres-
sure reaching the upper portion of the thoracic duct, eventually the left
bronchomediastinal trunk will be involved, at least in the instances in
which it empties directly into the thoracic duct. Although Korsch did
not specifically mention the right-sided paratracheal nodes, these could
be involved also if the disease lasts long enough, or if the obstruction
in the thoracic duct becomes sufficiently severe, by the opening of
anastomotic channels between right and left bronchomediastinal
trunks, and by subsequent backing up of accumulated fats through
these new channels.

Chylous ascites was present in our case, and in Blumgart's[2] second
case, as well as in those of Reinhart and Wilson,[5] and Sailer and Mc-
Gann.[7]

DISCUSSION

Attempts to explain the etiology and pathogenesis of intestinal lipodystrophy have been at least as numerous as the case reports; all of them, needless to say, are highly theoretical. In general, they fall into three groups: the first seeks to identify Whipple's disease with other previously described diseases; the second would incriminate a phase of fat digestion and absorption, either normal or abnormal; and the third evokes a mechanical or obstructive factor.

In the first group, sprue, local lipoid storage, and generalized lipoidoses have been enumerated most often. It is generally accepted, however, that Whipple's disease can be identified with none of these. In addition, it is worth mentioning that Whipple[1] observed in the fat vacuoles and foam cells of a mesenteric lymph node a rod-shaped body that stained only with the Levaditi technic, and that suggested to him a possible relationship between his case and the group of spirochetal diseases. Such a body has not been described since, and the positive serologic findings in Reinhart and Wilson's[5] case as well as in our own were probably entirely coincidental.

In the attempt to demonstrate that Whipple's disease is in effect an intestinal lipodystrophy, almost no phase of the mechanism of fat digestion and absorption has been neglected. It has even been suggested that the anatomic findings might represent merely a phase in normal alimentation; or of increased intake of fat or of peculiarities of diet with absorption of unusual fats. A deficiency in pancreatic lipase has been postulated. Pearse[10] incriminated bile salt metabolism. Apperly and Copley[12] felt that there was a failure in resynthesis with retention of fatty acids in the tissues. Glynn and Rosenheim,[15] whose patient clinically was thought to have Addison's disease, called attention to the evidence that adrenalectomy interferes with the phosphorylation of fats in the intestine and delays fat absorption. Finally, an increased excretion of fat into the intestines rather than a diminished absorption has been suggested.

There remains the third group of theories, which center around obstruction of the thoracic duct or of the mesenteric lymphatics. A congenital form of obstruction is unlikely in view of the age distribution of the known cases. In the few cases of intestinal lipodystrophy in which the thoracic duct was dissected, it was free of obstruction; whereas obstruction of the thoracic duct from various causes as demonstrated at autopsy or from experimental ligation in animals has not been accompanied by the characteristic morphologic changes of Whipple's disease. Furthermore, Whipple deduced, from the fact that fatty acid crystals and mast cells were present in the fluid of the duct,

that there was also no obstruction in the lymphatic radicles between the mesenteric nodes and the receptaculum. In our own case, and in others in which mediastinal and tracheobronchial nodes were involved, it seems a reasonable assumption that there was no complete obstruction, at least, either in the efferent lymphatics of the mesenteric nodes or in the thoracic duct, since apparently there was a backing up of the accumulated fatty deposits into the thoracic nodes. It must be admitted, however, that such circumstantial evidence may rule out a completely obstructive lesion, but not a partial obstruction.

Unfortunately, from all these theories we can conclude only that in Whipple's disease there exist both obstruction in the lymphatic drainage of the small intestine and interference with the absorption of fats. The crux of the matter now appears to be: which is the primary factor? If the essential disturbance is one of malabsorption, although the cause of this is still unexplained and although it may proceed from a variety of causes, Whipple's disease can be considered a distinct entity. If, on the other hand, the malabsorption is only secondary to obstruction of lymphatics, the latter also attributable to a variety of as yet unexplained inflammatory or sclerosing lesions, the entity ceases to exist. For the obstruction may be encountered in its most innocuous form incidentally at autopsy, as, for example, a xanthomatous deposit in the mesentery, thus substantiating Thannhauser's [17] theory; or, more conspicuously, as multiple cyst-like dilated lymph nodes, perhaps massive enough to have produced symptoms; or in its more malignant or end-stage, when the entire drainage of the intestinal lymphatics is partially or completely blocked.

SUMMARY

A case of intestinal lipodystrophy of Whipple is presented.

In an analysis of reports of similar cases, two not previously so designated have been included. The theoretical explanations of Whipple's disease fall into three groups: those which seek to identify it with other previously described diseases; those which implicate a phase of normal or abnormal fat digestion and absorption; those which invoke a mechanical or obstructive factor.

Attention is called to the possibility that Whipple's disease may not be a clinical or pathological entity.

REFERENCES

1. Whipple, G. H. A hitherto undescribed disease characterized anatomically by deposits of fat and fatty acids in the intestinal and mesenteric lymphatic tissues. *Bull. Johns Hopkins Hosp.*, 1907, 18, 382–391.
2. Blumgart, H. L. Three fatal adult cases of malabsorption of fat. *Arch. Int. Med.*, 1923, 32, 113–128.

3. Jarcho, S. Steatorrhoea with unusual intestinal lesions. *Bull. Johns Hopkins Hosp.*, 1936, 59, 275–286.

4. Hill, J. M. Mesenteric chyladenectasis. Report of a case. *Am. J. Path.*, 1937, 13, 267–275.

5. Reinhart, H. L., and Wilson, S. J. Malabsorption of fat (intestinal lipodystrophy of Whipple). Report of a case. *Am. J. Path.*, 1939, 15, 483–491.

6. Boeck, W. C. Discussion of: Bargen, J. A., Bollman, J. L., and Kepler, E. J. Diarrhea of pancreatic insufficiency. *Am. J. Digest. Dis.*, 1937–38, 4, 731.

7. Sailer, S., and McGann, R. J. Lipophagic granulomatosis of the enteric tract. *Am. J. Digest. Dis.*, 1942, 9, 55–63.

8. Fleischmann, R. Über tumorbildende Fettgewebsgranulome im Gekröse des Dünndarms. *Arch. f. klin. Chir.*, 1930, 158, 692–701.

9. Korsch, H. J. Fettstoffwechselstörung mit Granulombildung im Mesenterium. *Zentralb. f. allg. Path. u. path. Anat.*, 1938–39, 71, 337–344.

10. Pearse, H. E. Whipple's disease, or intestinal lipodystrophy. *Surgery*, 1942, 11, 906–911.

11. Collins, A. N., and Berdez, G. L. Chyle cysts of the mesentery. *Arch. Surg.*, 1934, 28, 335–344.

12. Apperly, F. L., and Copley, E. L. Whipple's disease (lipophagia granulomatosis). *Gastroenterology*, 1943, 1, 461–470.

13. Amsterdam, H. J., and Grayzel, D. M. Intestinal lipodystrophy (lipophagia granulomatosis or Whipple's disease). *Am. J. M. Sc.*, 1945, 210, 605–611.

14. Vaux, D. M. Chyladenectasis with steatorrhoea. *J. Path. & Bact.*, 1943, 55, 93–96.

15. Glynn, L. E., and Rosenheim, M. L. Mesenteric chyladenectasis with steatorrhoea and features of Addison's disease. *J. Path. & Bact.*, 1938, 47, 285–290.

16. Fitzgerald, P. J., and Kinney, T. D. Intestinal lipodystrophy (Whipple's disease). *Am. J. Path.*, 1945, 21, 1069–1089.

17. Thannhauser, S. J. Lipoidoses: Diseases of the Cellular Lipid Metabolism. Oxford University Press, New York, 1940, p. 252.

18. Bunting, C. H., and Huston, J. Fate of the lymphocyte. *J. Exper. Med.*, 1921, 33, 593–600.

19. Thompson, H. G. The lymphoid tissue of the alimentary canal. *Brit. M. J.*, 1938, 1, 7–11.

20. Erf, L. A. The disappearance of intravenously injected lymphocytes in the absence of the gastrointestinal tract. *Am. J. M. Sc.*, 1940, 200, 1–11.

21. Bloor, W. R. Biochemistry of the Fatty Acids and Their Compounds, the Lipids. Reinhold Publishing Corp., New York, 1943, p. 97.

[*Illustrations follow*]

DESCRIPTION OF PLATES

PLATE 75

FIG. 1. *a*. Cross section of wall of jejunum and mesentery. Dilated lymphatics in wall of jejunum and mesentery appear as white spots and streaks. Many of the irregular white areas in the mesenteric fat are xanthomatous foci. *b*. Mucosal surface of jejunum, showing prominent valvulae conniventes and plush-like appearance. The latter is due to enlargement of the villi, which are seen in the photograph as pale dots. *c*. Serosal surface of jejunum. The dilated lymphatics appear in part as a tracery of white lines, and in part as white spots. Some of the irregular white areas are xanthomatous foci.

FIG. 2. Circular fold of jejunum, showing enlarged, bizarre-shaped villi, homogeneous coagulum in stroma of villi, and in mucosal and submucosal lymphatics, and masses of foam cells in submucosa. $\times$ 25.

A   B   C
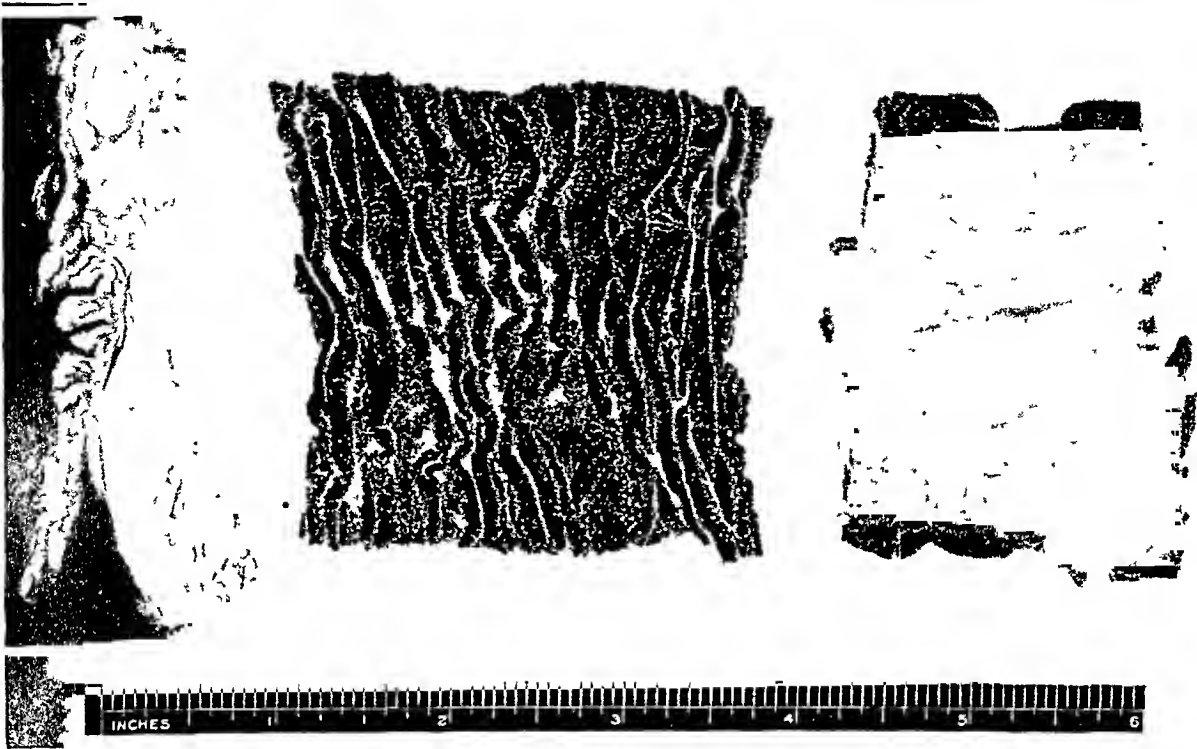
Rosen and Rosen

Intestinal Lipodystrophy

PLATE 76

Fig. 3. Mesentery. Dilated lymphatics filled with coagulum and foam cells. Accumulation of foam cells in the fat (xanthomatous focus). $\times$ 200.

Fig. 4. Tracheobronchial lymph node, showing dilated sinuses filled with coagulum and foam cells, and compressed lymphatic cords. $\times$ 50.

Fig. 5. Same lymph node as shown in Figure 4. $\times$ 400.

3

4

5

Rosen and Rosen

Intestinal Lipodystrophy

INFECTIOUS MONONUCLEOSIS
An Autopsy Report [*]

Fred H. Allen, Jr.,[†] Captain, M.C., A.U.S., and Aaron Kellner,[‡] Captain, M.C., A.U.S.

(From the Laboratory Services, AAF Station Hospital, Bradley Field, Conn., and AAF Regional Hospital, Westover Field, Mass.)

Infectious mononucleosis has come to be regarded as a fairly common disease, occurring sporadically or in small epidemics. The mortality from the disease has been almost negligible, with a resulting paucity of material for histopathologic study. The only previously reported case which was autopsied was that of Ziegler,[1] in which the patient died of a ruptured spleen in the fourth week. In this paper we wish to report the autopsy findings of a case of infectious mononucleosis in which the patient died accidentally in an airplane crash about 1 month after the onset of the acute illness.

The clinical entity of glandular fever was described by Pfeiffer [2] in 1889. In 1920 Sprunt and Evans [3] described "infectious mononucleosis," which a year later was shown to be identical with glandular fever by Tidy and Morley.[4] The heterophil or sheep cell agglutination was described by Paul and Bunnell [5] in 1932. This test, plus the description of the characteristic hematologic findings by Osgood,[6] Kracke and Garver,[7] and Downey and Stasney,[8] made possible accurate, objective laboratory diagnosis of infectious mononucleosis. The widespread use of these laboratory procedures, especially in the Armed Forces in which almost all patients with upper respiratory infections are hospitalized, has brought to light many cases of infectious mononucleosis which would ordinarily remain undiagnosed, and has led to the realization that it is a much more common disease than is generally appreciated.

The clinical features of infectious mononucleosis are protean, and the severity of the disease is extremely variable. The more common clinical picture includes fever, malaise, headache, sore throat, lymphadenopathy, splenomegaly, skin lesions, hepatomegaly, and gastrointestinal complaints. Jaundice during infectious mononucleosis has been observed in 3 to 5 per cent of cases. Involvement of the central nervous system associated with abnormalities in the spinal fluid has been reported by Epstein and Dameshek,[9] Thelander and Shaw,[10] and Landes, Reich, and Perlow.[11] Myocardial changes during acute infectious mononucleosis have been noted by Candel and Wheelock [12]

who reported a case with electrocardiographic evidence of acute myocarditis, and by Logue and Hanson [13] who reported a case with first degree heart block.

The reason for the wide diversity of clinical manifestations becomes readily apparent from the autopsy report of Ziegler [1] and from the case to be described below. Infectious mononucleosis is a generalized disease with organic changes, as evidenced by cellular infiltration in almost every organ in the body. The clinical picture is the composite of the changes wrought in the affected organs, and will vary in type and severity with the organ system predominantly involved. The lymph nodes, spleen, and nasopharyngeal tissues appear to be the more usual sites of involvement, but changes can occur in the lungs, liver, kidneys, skin, heart, testes, adrenals, brain, and probably in other organs; and thus account for the occasional atypical or unusual cases.

The cause of the disease is still uncertain. The concept that "Bacillus monocytogenes" is the etiologic agent is no longer tenable. The general opinion is that the etiologic agent is probably a virus, although no specific virus has as yet been isolated.

The histologic features of infectious mononucleosis have been studied from surgical material and that taken for biopsy. Sprunt and Evans [3] stated that the histopathologic picture was not distinctive and may suggest one of the lymphomas. Fox,[14] in 1927, studied a tonsil and cervical lymph node and concluded that they showed nothing specific—only hyperplasia of the lymphoid elements with retention of the normal architecture. Pratt,[15] in 1931, reported the findings in two biopsies of cervical lymph nodes removed from himself a year apart; and found marked reticulo-endothelial hyperplasia in the first specimen. The second showed a similar but less marked hyperplasia, with some fibrosis. Downey and Stasney [16] studied lymph nodes from 8 cases of infectious mononucleosis taken at various stages of the disease. They noted extreme hyperplasia of both the lymphocytes and the reticulum, but never complete obliteration of the nodal architecture. They also concluded from imprint studies that the atypical lymphocytes present in the peripheral blood had their origin in the nodes. Gall and Stout,[17] in 1940, studied lymph nodes removed from 10 patients, and described a morphologic pattern which they considered characteristic of the disease. They emphasized again that the nodal architecture was preserved, although it was distorted in some cases. They described three predominant features which distinguished infectious mononucleosis from ordinary hyperplasia: first, marked proliferative activity in the pulp which tended to obscure the margins of the follicles; second, focal proliferative activity of the clasmatocytes, which simulated "epithe-

lioid cells" and formed small nodules (no necrosis or giant cells were ever noted); and third, the presence in the nodes of many "infectious mononucleosis cells." These cells they described as large, with "abundant, slightly foamy, cerulean blue cytoplasm," identical on imprint preparation and supravital staining with the characteristic cells found in the circulating blood. These cells they considered almost pathognomonic of the disease, and seen best with Zenker's fixation and the phloxine-methylene blue stain. King,[18] in 1941, reported a case of spontaneous rupture of the spleen in infectious mononucleosis. Microscopic study of the spleen and appendix removed at laparotomy revealed nothing distinctive. Straus,[19] in 1942, reported a case in which an appendix, removed during acute infectious mononucleosis, showed morphologic changes in the lymphoid tissue identical with those seen in a lymph node removed at the same time, and similar to those described by Downey and Stasney, and Gall and Stout. Darley, Black, Smith, and Good [20] reported spontaneous rupture of the spleen in infectious mononucleosis. Examination of the spleen revealed an increase in lymphoid elements and the presence of an atypical cell similar to the characteristic lymphocytes found in the peripheral blood. An additional case of traumatic rupture of the spleen was reported by Milne.[21] The preliminary microscopic diagnosis was Hodgkin's disease, but further study revealed infectious mononucleosis.

Ziegler,[1] in his report of a fatal case with autopsy, described changes in the liver, kidneys, lungs, and spleen. The lesions in the liver, kidneys, and lungs consisted of focal infiltrations of mononuclear cells, with reticulocyte proliferation and necrosis. The changes in the spleen were more diffuse than focal in character.

REPORT OF CASE *

H. S., a white, American Army Air Forces pilot, 23 years old, was admitted to a small station dispensary on about May 11, 1945, because of malaise and fever. He was kept in bed, given two "sulfa" tablets four times a day, and penicillin for 1 day before being admitted to a station hospital on May 18. He had previously been in excellent health and physical condition. Family history and previous personal history were not significant. He had had no serious illness or injury.

At the time of admission to the station hospital he was complaining of headache, and was febrile. There was no cough. Physical examination was negative. The skin was clear, and there was no significant glandular enlargement. The lungs were clear; the heart, normal. There were no signs of disease of the nervous system. Blood taken at the time of admission showed a red cell count of 4,500,000; hemoglobin, 80 per cent (Sahli); 11,450 white blood cells with 14 per cent neutrophils, 77 per cent lymphocytes, 9 per cent monocytes. The urine was negative.

A roentgenogram of the chest taken on the day after admission showed a definite increase in the vascular and peribronchial markings on the right side. The hilar

* The clinical record was obtained through the courtesy of Capt. William C. Weir, M.C.

markings were also increased in prominence. In the lower aspect of the right lung an early peribronchial infiltration was noted. These findings suggested the diagnosis of primary atypical pneumonia.

Blood taken on May 22, 4 days after admission and about 11 days after the onset of the illness, showed a heterophil antibody titer of 1:896. On May 25, he complained of sore throat, was found to have a red and edematous pharynx, and was given 50,000 units of penicillin with marked improvement in the following 2 days. He was asymptomatic and afebrile after May 27.

On May 28, the white blood cell count was 10,000, with 30 per cent neutrophils and 61 per cent lymphocytes. A further report (undated) gave a differential count of 13 per cent neutrophils, 83 per cent lymphocytes, 3 per cent monocytes, and 1 per cent eosinophils, with the remark that the lymphocytes were atypical and characteristic of infectious mononucleosis. The heterophil antibody test was repeated on May 28, and the titer was again found to be 1:896.

Roentgenograms of the lungs on May 31 showed no abnormalities. The patient was discharged to duty on June 1. On June 10, approximately 1 month from the onset of illness, and 2 weeks after the remission of clinical symptoms, he crashed while piloting an airplane, and was dead when pulled from the plane a few minutes later. Autopsy was performed 30 hours after death.

AUTOPSY FINDINGS

The gross findings were essentially those of severe trauma. There were multiple fractures of the skull and facial bones, and compound fractures of the left femur and right hand. There was a moderate amount of subdural and subarachnoid hemorrhage, and occasional petechial hemorrhages were present in the brain substance. There was also extensive pulmonary hemorrhage. The liver, spleen, kidneys, and remaining viscera were of normal size and weight, and showed no gross abnormalities, with the exception of the retroperitoneal and hilar lymph nodes, which were discrete and grossly enlarged, measuring up to 3 cm. in greatest dimension.

Microscopic Examination

All tissues except the brain were fixed in Zenker's solution; the brain was fixed in 10 per cent formalin. All sections were stained with hematoxylin and eosin.

Liver. The liver parenchyma was studded with small, discrete, focal areas of cellular infiltration (Figs. 1 and 2). Many of these infiltrates were perilobular in distribution, but just as many were scattered through the lobules with no characteristic localization. The cellular infiltrations consisted almost exclusively of rather large mononuclear cells, with oval or rounded nuclei, a few of which were reniform. Varying numbers of lymphocytes, and an occasional neutrophil, were present. Definite vacuolization of the cytoplasm of the mononuclear cells could not be made out. In the areas of infiltration the liver cells had mostly disappeared. The liver cells immediately surrounding these areas showed some degree of atrophy and no evidence of regeneration.

Kidney. Scattered throughout the renal cortex and medulla were many small, focal areas of mononuclear infiltration similar to those described above. There were atrophy, degeneration, and disappearance of the tubules in some of these areas (Fig. 4).

Heart. The heart showed a few interstitial collections of mononuclear cells and lymphocytes (Fig. 5). These collections were small to moderate in size. There was no atrophy or replacement of the muscle fibers.

Lung. There were many red blood cells, considerable edema fluid, and the usual numbers of "heart lesion" cells in the alveoli and bronchi. Many nodular collections of mononuclear cells and lymphocytes, similar to those previously described, were seen (Fig. 3). These were in relation to bronchi and blood vessels, and often within the interstitial tissues. Anthracotic pigment was present in the usual quantities in the peribronchial lymphoid tissue, but was not present in the nodules.

Testis. A moderate number of focal collections of mononuclear cells were seen within the interstitial tissue of the testis and the tunica albuginea (Fig. 6). These were quite large and were morphologically similar to those previously described. Spermatogenesis and the cells of Leydig were within normal limits.

Adrenal. There were a few foci of mononuclear infiltration present chiefly in the adrenal medulla, occasionally in the cortex and capsule (Fig. 7). These were not too unlike the lymphoid collections often found in this organ, and may not have been the result of infectious mononucleosis.

Brain. An occasional blood vessel in the cerebral cortex showed heavy cuffing of mononuclear cells, mostly lymphocytes (Fig. 8).

Spleen. The splenic architecture, follicles, and pulp were essentially normal. Moderate numbers of eosinophils were present, consistent with the sudden death. The sinusoids were prominent, and there was some proliferation of reticulo-endothelial cells.

Lymph Nodes. The nodal architecture was preserved. The sinusoids were unusually prominent, but aside from a moderate degree of hyperplasia of the reticulo-endothelial cells there was no striking abnormality.

Other Organs. Sections of aorta, pancreas, esophagus, pylorus, jejunum, ileum, appendix, urinary bladder, prostate, diaphragm, thymus, pituitary body, and costal bone marrow showed no abnormalities except for post-mortem autolytic changes. However, it is possible that if larger amounts of tissue had been preserved and more sections taken for study, additional foci of cellular infiltration might have been encountered.

COMMENT

This patient had clinical infectious mononucleosis 2 to 4 weeks prior to death. The diagnosis was established beyond reasonable doubt by the clinical picture, hematologic findings, and the strongly positive heterophil antibody test on two separate occasions. It is therefore logical to assume that the microscopic lesions noted are those of a late stage of infectious mononucleosis. They are very similar in type and distribution to the visceral lesions described by Ziegler.[1] Unfortunately, the body was not obtained for autopsy until 30 hours after death, and had not been refrigerated during relatively hot weather. The tissue changes which ensued obscured the precise histologic details of the lesions. In addition, infectious mononucleosis was not suspected at the time of autopsy, and it was not until several weeks later that the clinical record was obtained. Had we been aware that the patient had just recovered from acute infectious mononucleosis, more careful search for lymph nodes would have been made, and special studies, such as imprint smears and supravital stains, would have been attempted.

This case emphasizes again the basic concept suggested by Ziegler [1] and others that infectious mononucleosis is a generalized disease, with lesions in many, and possibly all, organs of the body. The lesion consists essentially of a focal infiltration of mononuclear cells, including variable numbers of small lymphocytes, which in some cases crowds out and replaces the normal parenchyma. No evidence of fibrosis of the lesions was noted. It may be that the mononuclear cells are identical with the atypical lymphocytes seen in the peripheral blood and described by Gall and Stout [17] in affected lymph nodes, but we could not ascertain this from our sections.

Focal microscopic infiltrations were seen in the liver, kidneys, heart, lungs, testes, adrenals, and brain. The lesions in the adrenals are somewhat questionable, as similar collections of lymphoid tissue are frequently encountered in this organ. It is quite remarkable that the lymph nodes and spleen, which are usually most profoundly affected in infectious mononucleosis, were in this case relatively uninvolved. It is possible that in this particular case these organs were less involved than usual, or that they returned to normal more rapidly than other tissues.

The lungs are of considerable interest in this case. On the basis of the roentgenologic evidence, a tentative diagnosis of "primary atypical pneumonia, etiology unknown" was made—the nomenclature used in the Army to label so-called "virus" pneumonia. Since this patient did have definite clinical infectious mononucleosis at the time the roent-

genogram was made, it is possible that the nodular infiltrations in the lungs were those of infectious mononucleosis, rather than of a less likely co-existing independent disease involving the respiratory tract. It may very well be that infectious mononucleosis can produce a clinical and radiographic picture simulating "virus" pneumonia, as has been suggested by Halcrow, Owen, and Rodger.[22]

The brain in this case presented the first opportunity, to our knowledge, to study sections of the central nervous system in infectious mononucleosis. As mentioned previously, clinical involvement of the central nervous system, with spinal fluid changes, has been reported several times. Histologic study of the brain in this case, which had no clinical symptoms referable directly to organic changes in the central nervous system, revealed a few blood vessels, chiefly in the cortex, with perivascular infiltrations of lymphoid tissue. The histologic picture was similar to that seen in mild virus encephalitis. It thus appears likely that the central nervous system shares with the other viscera the generalized involvement in infectious mononucleosis.

Histologic changes in the heart in infectious mononucleosis have not been described previously, but have been postulated on the basis of electrocardiographic findings. The interstitial infiltrations seen in the heart in this case are compatible with conduction changes demonstrable by electrocardiograms, assuming that the infiltrations may occur in any part of the cardiac muscle and may involve important conduction fibers.

The presence of microscopic infiltrations in the liver and kidneys furnishes the organic background to explain those occasional cases of infectious mononucleosis in which "hepatitis" or "nephritis" dominates the clinical picture. It is probable, due to the diffuse nature of the disease, that these organs are involved to some degree in almost every case, although rarely with sufficient alteration of function to be clinically detectable.

SUMMARY

Necropsy was done in a case of infectious mononucleosis, in which the patient had died 2 to 4 weeks after the acute illness as a result of an accident. Focal cellular infiltrations, similar to those previously described in infectious mononucleosis, were found in the liver, kidneys, heart, lungs, adrenals, testes, and brain. Cerebral and cardiac lesions, not previously described, were found.

This case emphasizes again that infectious mononucleosis is a generalized disease, and may produce organic changes in many viscera, thus explaining the wide diversity of clinical manifestations which are encountered.

It is suggested that infectious mononucleosis may produce a clinical and radiographic picture simulating "primary atypical pneumonia."

We are indebted to Mr. John Carabitses, Children's Hospital, Boston, for the photomicrographs.

REFERENCES

1. Ziegler, E. E. Infectious mononucleosis. Report of a fatal case with autopsy. *Arch. Path.*, 1944, 37, 196–201.

2. Pfeiffer, E. Drüsenfieber. *Jahrb. f. Kinderh.*, 1889, 29, 257–264.

3. Sprunt, T. P., and Evans, F. A. Mononuclear leukocytosis in reaction to acute infections ("infectious mononucleosis"). *Bull. Johns Hopkins Hosp.*, 1920, 31, 410–417.

4. Tidy, H. L., and Morley, E. B. Glandular fever. *Brit. M. J.*, 1921, 1, 452–456.

5. Paul, J. R., and Bunnell, W. W. The presence of heterophile antibodies in infectious mononucleosis. *Am. J. M. Sc.*, 1932, 183, 90–104.

6. Osgood, E. E. Fenestration of nuclei of lymphocytes: a new diagnostic sign in infectious mononucleosis. *Proc. Soc. Exper. Biol. & Med.*, 1935–36, 33, 218–219.

7. Kracke, R. R., and Garver, H. E. Diseases of the Blood and Atlas of Hematology. J. B. Lippincott Co., Philadelphia, 1937, ed. 1.

8. Downey, H., and Stasney, J. Infectious mononucleosis. Part II. Hematologic studies. *J. A. M. A.*, 1935, 105, 764–768.

9. Epstein, S. H., and Dameshek, W. Involvement of the central nervous system in a case of glandular fever. *New England J. Med.*, 1931, 205, 1238–1241.

10. Thelander, H. E., and Shaw, E. B. Infectious mononucleosis with special reference to cerebral complications. *Am. J. Dis. Child.*, 1941, 61, 1131–1145.

11. Landes, R., Reich, J. P., and Perlow, S. Central nervous system manifestations of infectious mononucleosis. Report of a case. *J. A. M. A.*, 1941, 116, 2482–2484.

12. Candel, S., and Wheelock, M. C. Acute non-specific myocarditis. *Ann. Int. Med.*, 1945, 23, 309–337.

13. Logue, R. B., and Hanson, J. F. Heart block: a study of 100 cases with prolonged P-R interval. *Am. J. M. Sc.*, 1944, 207, 765–769.

14. Fox, H. Infectious mononucleosis. II. Histology of a tonsil and a lymph node. *Am. J. M. Sc.*, 1927, 173, 486–489.

15. Pratt, C. L. G. The pathology of glandular fever. *Lancet*, 1931, 2, 794–795.

16. Downey, H., and Stasney, J. The pathology of the lymph nodes in infectious mononucleosis. *Folia haemat.*, 1935–36, 54, 417–438.

17. Gall, E. A., and Stout, H. A. The histological lesion in lymph nodes in infectious mononucleosis. *Am. J. Path.*, 1940, 16, 433–448.

18. King, R. B. Spontaneous rupture of the spleen in infectious mononucleosis. Report of a case. *New England J. Med.*, 1941, 224, 1058–1060.

19. Straus, R. Infectious mononucleosis simulating acute appendicitis with description of a specific lesion of the appendix. *Am. J. Clin. Path.*, 1942, 12, 295–301.

20. Darley, W., Black, W. C., Smith, C., and Good, F. A. Spontaneous splenic rupture in infectious mononucleosis; a case and a pathologic report. *Am. J. M. Sc.*, 1944, 208, 381–384.

21. Milne, J. Infectious mononucleosis. *New England J. Med.*, 1945, 233, 727–731.

22. Halcrow, J. P. A., Owen, L. M., and Rodger, N. O. Infectious mononucleosis, with an account of an epidemic in an emergency medical service hospital. *Brit. M. J.*, 1943, 2, 443–447.

DESCRIPTION OF PLATES

PLATE 77

All sections were stained with hematoxylin and eosin. $\times$ 120 except for Figure 1.

FIG. 1. Liver, high power. Focal mononuclear infiltration, with replacement of liver cells. $\times$ 450.

FIG. 2. Liver, low power. Focal collections of mononuclear cells.
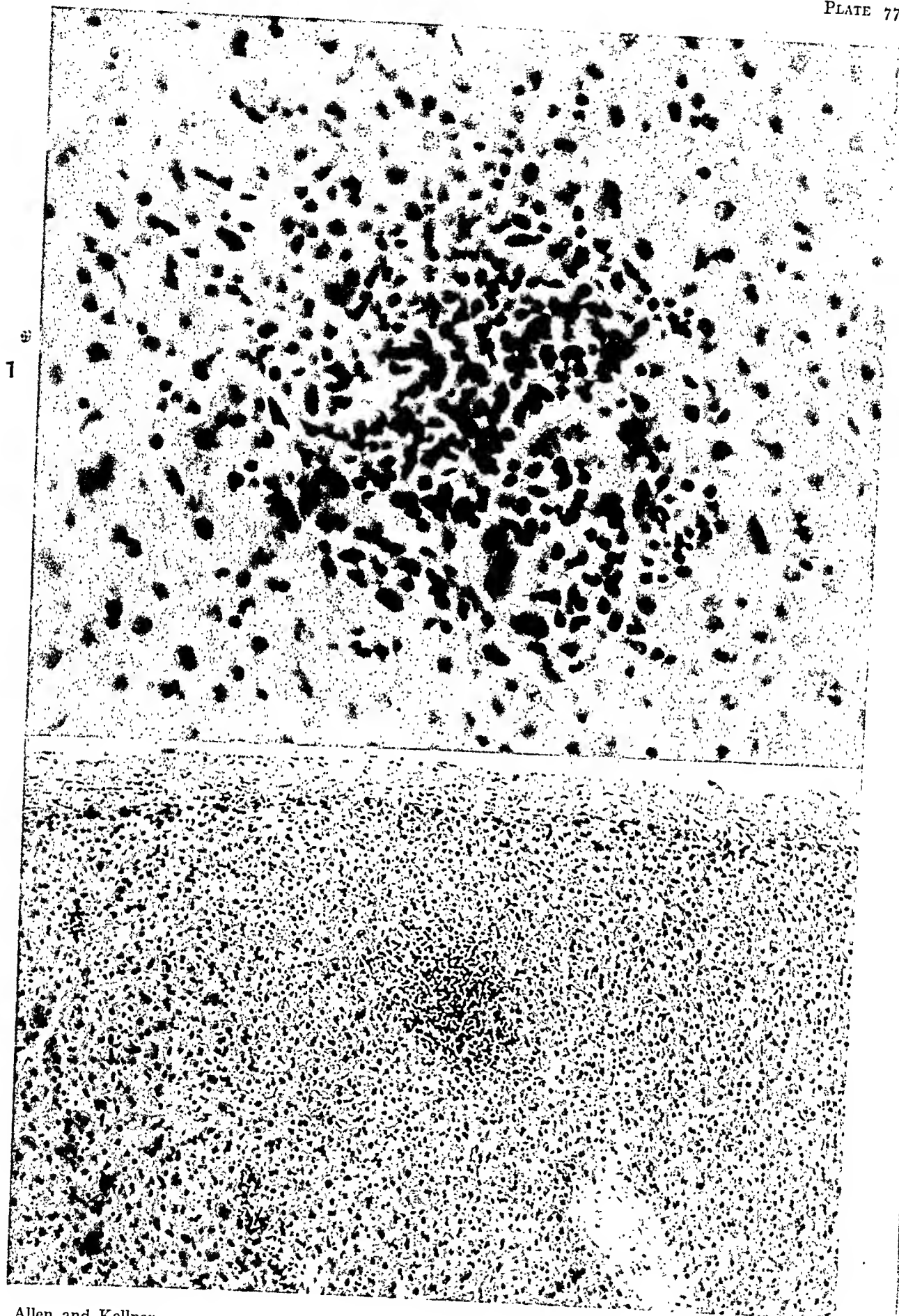
PLATE 77

Allen and Kellner

Infectious Mononucleosis

PLATE 78

PLATE 79

FIG. 6. Testis, low power. Focal interstitial infiltration of mononuclear cells.

FIG. 7. Adrenal, low power. Focal infiltration of mononuclear cells in medulla.

FIG. 8. Brain, low power. Cortical vessel with perivascular cuff of mononuclear cells.
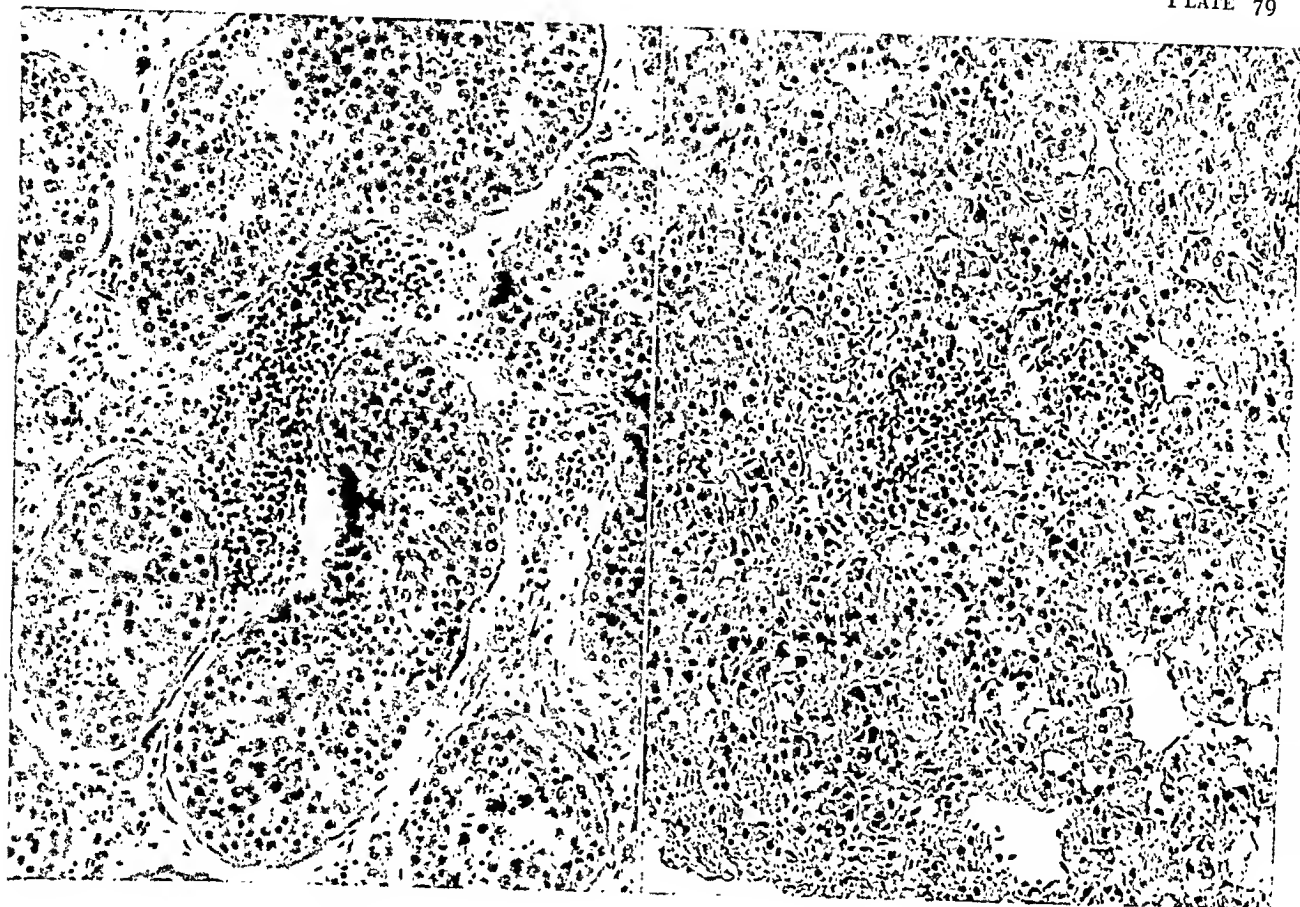
6

7
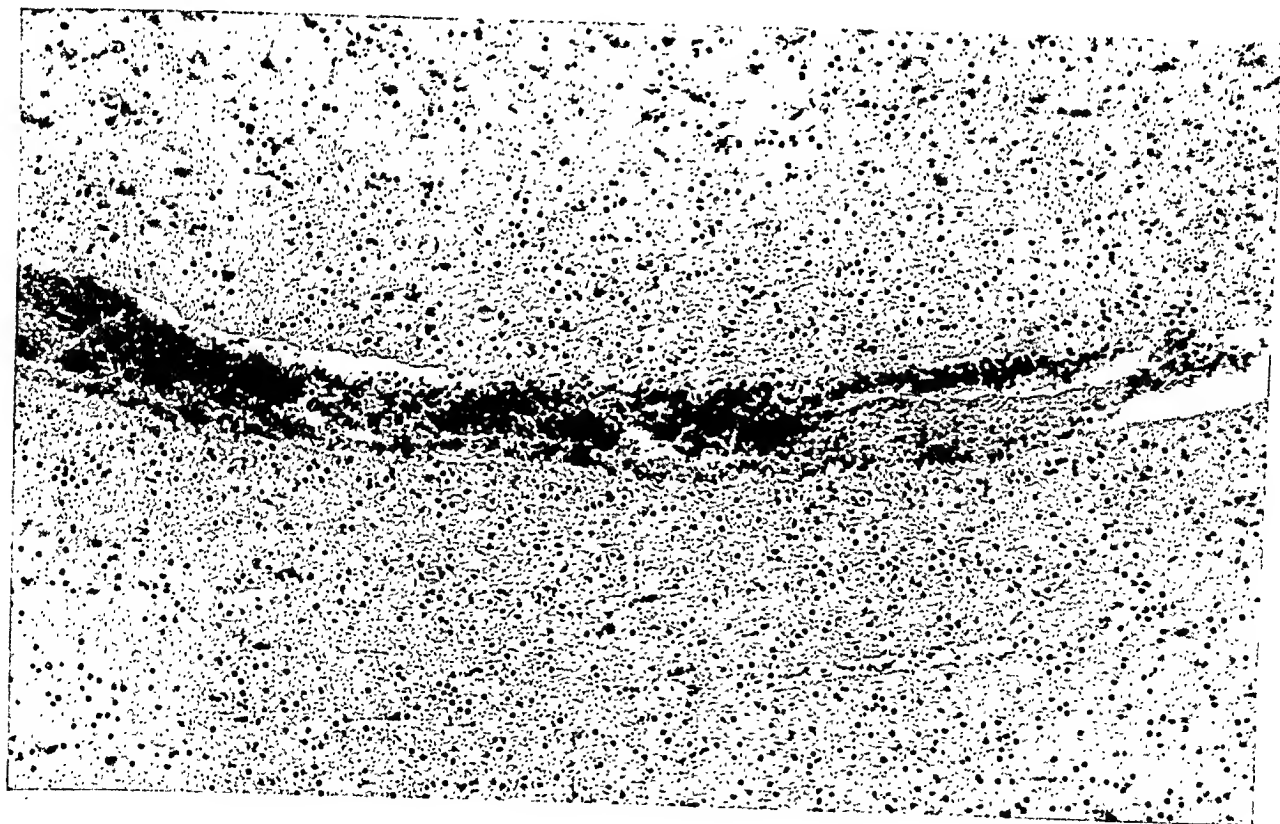
8

Allen and Kellner

Infectious Mononucleosis

CYSTS OF THE ADRENAL GLAND WITH CASE REPORT *

Dexter L. Reimann, M.D., and William L. Guyton, M.D.

(*From the Department of Pathology, School of Medicine, University of Maryland, Baltimore 1, Md.*)

In the past 20 years only four papers on adrenal cysts have appeared in American literature. As there is a scarcity of material on this subject, this report is believed to be justified.

The mechanism by which adrenal cysts are formed does not coincide with the manner in which true retention or inclusion cysts are developed. They result usually from ectasia of pre-existing vessels [1] or cystic degeneration of hematomas or adenomas.[2] Therefore, they are better called pseudocysts.

A classification of these lesions by Levison [3] includes true glandular cysts, cystic adenomas, cystic lymphangiomas, pseudocysts, and echinococcus cysts. The first two classes are excluded from this paper because satisfactory reports and descriptions were not found in the literature. Echinococcus cysts of the adrenal gland are exceedingly rare. From a study of 1,617 cases of echinococcosis by Barnett,[4] it was shown that the adrenal gland is involved in much less than 0.5 per cent. Dew,[5] in his exhaustive monograph, cited only a single case of echinococcus disease involving the adrenal gland and in this the lesion occupied a position in the capsule.

It also has been suggested that cysts of the adrenal glands are of a secondary nature. According to Rabson and Zimmerman,[1] cystic lymphangiomas of the adrenal gland should be classed more accurately as lymphangiectasias. They concluded that lymphangiomas and hemangiomas are rare in the suprarenal body and that hamartomas are the most common cystic lesions seen in the adrenal gland. In this category the cases of Ballance [6] and of Rabson and Zimmerman may be included. Degeneration of hematomas resulting in pseudocyst formation was exemplified by the cases of Iglitsyn,[2] Pearse,[7] and most probably by that of Levison.[3]

Cysts of the suprarenal gland manifest themselves clinically in insidious and varied ways. That in the case of Rabson and Zimmerman [1] and the one recorded in this paper were found at autopsy and had apparently caused no disturbances during life. In the case reported by Ballance,[6] the patient, a 49-year-old white female, complained of sense of pressure, "indigestion," and back pain which passed around to the epigastrium. This complaint had existed for 5 years. The cyst in this case was externally palpable. Acute abdominal pain, shock, and palp-

able tumor which became apparent over a 3-day period were seen in a case reported by Pearse.[7] An erroneous diagnosis of "gallstones" and "pleurisy" had been made in this case. Levison's [3] patient manifested a pluriglandular syndrome which led to impressions of parathyroid and thyroid dysfunctions.

The gross appearance of adrenal cysts varies as does the pathogenesis. Those cysts resulting from ectasia of lymph channels consist of numerous locules that contain clear or milky fluid. The compartments reportedly may vary from 1 to 13 mm.[1] Cysts measuring 23 by 15 cm. and containing 1¼ liters of fluid have been reported.[6] The walls of the lymphangiectatic cysts are delicate and smooth.

The hematocysts have irregularly pigmented walls which may contain foci of calcareous material.[3] This is true whether the hematocysts develop in normal adrenal gland or adenomatous areas. Cysts of this type may contain bloody or reddish brown fluid. Ballance [6] cited the case of Doran in which the cyst contained 250 cc. of bloody fluid and that of Hartwell in which the cyst contained 3 liters of reddish brown fluid.

The following case is an example of lymphangiectasia of the adrenal gland.

REPORT OF CASE

M. R. (U. H. no. 86984), was a white female, 58 years of age, who was admitted with complaints of generalized abdominal pain, nausea, and vomiting of 8 days' duration. Physical and roentgenologic examinations of the abdomen led to a correct diagnosis of periappendicular abscess with partial intestinal obstruction. The chemical findings in the blood were normal with the exception of a decreased plasma chloride level and moderate hypoproteinemia. There was no clinical evidence of endocrinopathy.

Operative drainage of the abscess was undertaken. Postoperatively, the patient continued to suffer the effects of peritonitis. Treatment with penicillin and sulfonamide compounds effected no improvement. The patient died on the 14th postoperative day.

The findings at autopsy were consistent with the clinical diagnosis. The cystic right adrenal gland could in no way be related to the antemortem condition of the patient. The left adrenal gland was normal.

The right suprarenal body measured 7 by 2.4 cm. Cysts were externally apparent. On section the medulla was seen to contain smooth-walled cysts which ranged from 1 to 13 mm. in diameter. The fluid within the cysts was clear when fresh but became opaque and jelly-like in 10 per cent formaldehyde. There was no gross evidence of hemorrhage. The cysts had obviously distended the overlying cortex. Outside the walls of the larger cysts bits of compressed adrenal cortex were seen.

Microscopically, the cysts appeared as widely dilated spaces lined by

endothelium and filled with acellular, eosinophilic albuminous precipitate. Connective tissue separated the cysts. Calcareous deposits were present in the fibrous areas. The surrounding adrenal cortex showed pressure atrophy. In the better preserved portions of the cortex, glomerular, fascicular, and reticular zones could be discerned. The cortical cells were pale, granular, shrunken, and poor in lipoid material.

SUMMARY

Cysts of the adrenal glands are exceedingly rare. The most commonly reported types are hematocysts and lymphangiectatic cysts. A case of lymphangiectatic cysts of the right adrenal gland is reported.

REFERENCES

1. Rabson, S. M., and Zimmerman, E. F. Cystic lymphangiectasia of the adrenal. *Arch. Path.*, 1938, 26, 869–872.
2. Iglitsyn, N. M. [Blood cysts of suprarenals.] *Khirurgiya*, 1937, no. 1, 115–121.
3. Levison, P. A case of bilateral adrenal cysts. *Endocrinology*, 1933, 17, 372–376.
4. Barnett, L. Hydatid cysts: their location in the various organs and tissues of the body. *Australian & New Zealand J. Surg.*, 1942–43, 12, 240–248.
5. Dew, H. R. Hydatid Disease, Its Pathology, Diagnosis and Treatment. The Australasian Medical Publishing Co., Ltd., Sydney, 1928, p. 406.
6. Ballance, H. A. Cyst of the right suprarenal capsule removed by operation. *Brit. M. J.*, 1923, 1, 926–928.
7. Pearse, H. E. Cysts of the adrenals: report of a case. *Tr. West. S. A.*, 1916, 26, 329–336.

[*Illustrations follow*]

DESCRIPTION OF PLATE

PLATE 80

FIG. 1. This photograph shows cross sections of the lymphangiectactic cystic adrenal. The largest cyst is seen in the upper pole of the adrenal gland pictured on the left. Many smaller cysts filled with mucinous material are seen in the midportion, and normal adrenal cortex is seen in the lower portion of the same section.

FIGS. 2 and 3. Endothelium-lined spaces filled with precipitated albuminous material are shown with recognizable adrenal cortex. Hematoxylin and eosin stain. × 100.
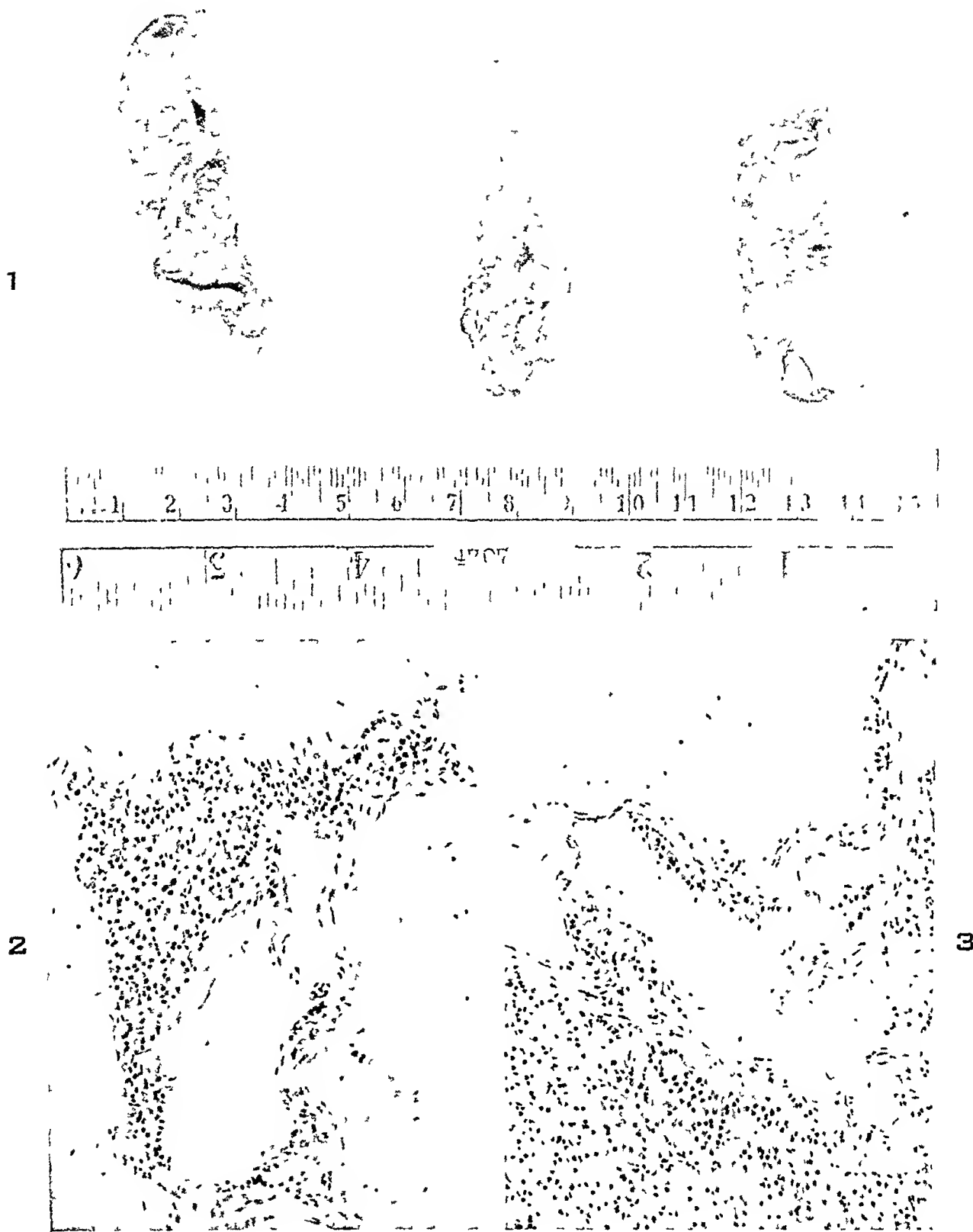
Reimann and Guyton

Cysts of the Adrenal Gland

THE PATHOGENESIS OF POLYCYSTIC PANCREAS
RECONSTRUCTION OF CYSTIC ELEMENTS IN ONE CASE *

ROBERT F. NORRIS, M.D.,† and RALPH M. TYSON, M.D.

(*From the Ayer Clinical Laboratory and the Pediatric Service, Pennsylvania Hospital, Philadelphia, Pa.*)

Polycystic disease of the pancreas has not been recognized at autopsy so frequently nor studied so widely as polycystic disease of the kidney. Although variation in the character and distribution of the lesions is great, "fibrocystic disease" of the pancreas, as it is often called in the pediatric literature, is a well recognized entity in infancy. Essentially the pancreatic ducts are characteristically distorted and segmented. Some of the segments are atrophic, others are dilated and cystic. Many of these cysts may be isolated. The main pancreatic duct may or may not be atretic. Much of the glandular tissue is atrophic or is replaced by proliferating fibrous tissue and scar tissue accompanied by varying amounts of acute and chronic inflammatory exudate. The amount of fibrous stroma is often greater than is the case in polycystic disease of other organs. Squamous metaplasia of the ducts of varying degrees is often observed. Although some of the ducts are dilated and cystic, the disease is rarely characterized by diffusely distributed and large cysts. For this reason, it has not always been clear to authors reporting such cases that the disease is similar to polycystic disease of other organs. Yet the frequent association of cystic fibrosis of the pancreas with polycystic disease of the kidney, liver, and lung indicates that the lesions are essentially identical in etiology if not in morphology.

When the lesions are manifest in infancy, steatorrhea and malnutrition are often conspicuous symptoms, and are evidently the result of deficiency or absence of the external secretion of the pancreas. Since the disturbances of metabolism are usually profound and fatal, the early death of most of these children probably accounts for the rarity of extensive polycystic lesions of the pancreas in adults.

The clinical importance of polycystic disease of the pancreas was reviewed by Andersen,[1,2] and additional reports more recently have been made by Rauch, Litvak, and Steiner,[3] Oppenheimer,[4] Robbin and Bernhard,[5] Wolman,[6] Daniel,[7] Snelling and Erb,[8] Kennedy and Baggenstoss,[9] and Menten and Middleton.[10] Although the etiology of the

lesions is discussed, these papers are concerned more with the clinical aspects of the disease and the frequently associated cystic lesions of the lung than with pathogenesis. In the past, however, careful microscopic studies to determine the cause of the lesions have been made, but agreement on the pathogenesis is lacking. Reviews of the earlier literature may be found in the papers of Sears,[11] Teuscher,[12] Bartoli,[13] Rümler,[14] and Pazzagli.[15]

In general, like polycystic liver, the polycystic lesions of the pancreas have not been so thoroughly studied by means of models as have those in the kidney. The various theories of causation suggested for polycystic kidney have also been proposed for the congenital lesions of the pancreas. By some, therefore, it is held that chronic inflammation and proliferation of fibrous tissue is the fundamental lesion and causes obstruction, segmentation, and cystic dilatation of the pancreatic ducts. Although inflammation is almost always present in the polycystic pancreas, there are many cases of polycystic liver and kidney in which it is absent. By others it is assumed that failure of the two anlagen of the pancreas properly to unite may cause obstruction of the proliferating ducts and cystic dilatation of the segments. Functionally and anatomically, however, these anlagen are not analogous with those of the kidney. In addition, the theory fails to explain the occurrence of polycystic lesions of the liver and lung which are not formed by the fusion of separate anlagen. Finally, the most commonly held theory among the older German writers is the concept of Albrecht[16] that an abnormal tumorlike proliferation of epithelial and fibroblastic tissue results in faulty development of the organ. Although in many instances of polycystic disease the epithelium and fibrous stroma may have the appearance of excessive proliferation, there is nothing characteristically neoplastic about the tissue.

A different approach to the problem of polycystic disease was that of Kampmeier[17-20] and McKenna and Kampmeier[21,22] who demonstrated that the first generations of nephrons in the normal kidney are provisional and who suggested that persistence of these provisional elements would explain the occurrence of polycystic kidneys. Recently, cystic disease of the kidney and liver were studied by Norris and Herman[23,24] and Norris and Tyson,[25] respectively. By means of serial sections and reconstructions it was demonstrated that the general development of organs in the presence of polycystic disease is remarkably normal and continued normal differentiation of many epithelial elements occurs simultaneously with distortion, segmentation, and cystic dilatation of others which are fully formed. This observation suggests that a "hamartoma" is not the fundamental cause and that defects occur only after the structural units of an organ are differentiated. In the kidney

it was not determined whether the segmentation was confined to the collecting ducts or included the uriniferous tubules as well. In the liver, however, only the small intrahepatic bile ducts were segmented and cystic. It was concluded that, since early generations of nephrons and intrahepatic bile ducts normally become segmented and then are resorbed, polycystic disease is an extension of this normal process of degeneration which includes a greater number of elements than normally. Instead of complete resorption, however, many of the epithelial segments persist to form the isolated cysts of polycystic disease. The cause of this abnormality is still problematical.

In continuance of the study of polycystic disease, we have reconstructed elements of a cystic pancreas in order to determine whether this theory is also applicable to other organs. This pancreas is from case 2 previously reported by Norris and Herman [24] and Norris and Tyson [25] in which case the kidneys and liver were also cystic. Although there were no dilated cysts in the kidney, liver, or pancreas, this case has been selected as previously indicated because it is believed that the lesions represent an early stage of polycystic disease and that a proper evaluation of the essential defect can be obtained only by a study of the disease in its incipiency.

MATERIALS AND METHODS

Briefly, the patient was a full-term infant who died 24 days after birth following the onset of a hemorrhagic diathesis, jaundice, and evidence of renal failure. Sixth digits of both hands were amputated after delivery. In the preceding papers [24,25] the clinical history, anatomic diagnosis, and pathologic findings in the kidneys and liver were described and will not be repeated. Only the gross and microscopic lesions of the pancreas will be presented at this time.

The tissues were fixed in Kaiserling's and Regaud's solutions. Blocks were embedded in paraffin and sections were stained with Delafield's hematoxylin and eosin. Sections for ordinary study were cut at 5 μ. In addition, several hundred serial sections, 15 μ in thickness, were cut at right angles to the long axis of the pancreas from three blocks about 2 cm. on a side. These were taken from the head, body, and tail, respectively. The cystic pancreatic ducts were traced and studied microscopically in the serial sections and some of them were reconstructed by the method previously described.[23]

GROSS EXAMINATION

At autopsy the pancreas was not weighed or measured but was described as uniformly enlarged. The contours were entirely normal and the position of the organ in the body was normal. On section, the main

pancreatic duct and its orifice were not identified. Only scattered, small, irregular, tubular and cystic ducts were present in the stroma. The parenchyma was fibrous, pale, and slightly mottled. There were no large cysts.

Microscopic Examination

In blocks from the head, body, and tail, the microscopic findings were similar. There were scattered lobules of acini, which were normally formed and were frequently located at the periphery of the pancreas. Individually the epithelial cells were smaller than is normal and appeared atrophic. Nearly all of the small pancreatic ducts in these areas were irregular and slightly dilated. Many of them contained inspissated débris resembling coagulated protein. The ducts were lined by single layers of flattened or cuboidal epithelium (Fig. 1). The epithelium of some of the ducts was duplicated and some showed varying degrees of squamous metaplasia. In other areas, the ducts were not associated with acini and were completely surrounded by dense fibrous stroma (Fig. 2). Invariably these ducts were distorted and irregularly dilated. The main pancreatic duct was not identified. The extent of these areas may be more readily appreciated in a low-power photomicrograph (Fig. 3). Few islets of Langerhans were seen. Those which were present were normally formed, but also appeared shrunken and atrophic. Like some of the ducts, many of them were isolated from any glandular tissue and were embedded in fibrous stroma. All of them, however, were in the vicinity of pancreatic ducts (Fig. 4). The fibrous stroma was compact and rarely was associated with fatty tissue except about the periphery of the pancreas. Blood vessels were numerous but were arranged in no definite pattern. Inflammatory exudate was scanty. Only a few lymphocytes, plasma cells, and mononuclear phagocytes were seen and these were usually scattered (Fig. 2).

In serial sections, the larger ducts varied greatly in contour and diameter. Although there were numerous zones of constriction between areas of dilatation, most of the ducts extended for considerable distances before ending blindly. There were, however, numerous blindly-ending outpocketings approximately at right angles to the main axes of the larger ducts. Some of these were pointed, others were blunt and bulbous. Many had multiple blindly-ending branches. These resembled branches of the larger ducts, but were atypical in arrangement and were irregularly distributed. Near the larger ducts were many completely isolated but undilated cysts which appeared to be pinched-off segments of the smaller branches of the ducts (Fig. 2). A segment of one of the larger ducts showing the characteristics described has been reconstructed and is illustrated in the model (Fig. 5).

DISCUSSION

According to Lewis,[26] the human pancreas is normally formed from two separate anlagen, the ventral and dorsal pancreases, which arise from the duodenum and which are present in embryos of 3 to 4 mm. They are still separated at 10 mm. by the portal vein, but at 16 mm. are united and partly surround the vein. With the elongation of the common bile duct, the ventral pancreas becomes completely separated from the duodenum and forms part of the head and much of the uncinate process. The dorsal pancreas forms the rest of these structures and the entire body and tail. The main ducts of the dorsal and ventral pancreases unite by a single anastomosis and only rarely are there any anastomoses between their branches. The main duct of the mature pancreas, formed by this anastomosis, empties into the common bile duct and is called the duct of Wirsung. The proximal portion of the duct of the dorsal pancreas, which arises from the duodenum, may persist as an accessory duct and is called the duct of Santorini. At first the newly formed main duct is a simple wide and hollow tube having numerous radial, pear-shaped buds and branches. These become canalized and continue to subdivide. The glandular epithelium is later differentiated into mature acini. The islets of Langerhans appear in the body and tail at 54 mm. but are not present in the head until a later period. At first they are connected with the ducts by epithelial stalks. In later stages they become detached from the epithelial tubes and remain isolated thereafter.

It is not clear whether early generations of pancreatic ducts are normally provisional as are early generations of nephrons and intrahepatic bile ducts. Since isolation of epithelial ducts by segmentation is a normal process of embryologic degeneration in other organs, it is quite possible that this process may at times occur in the normal fetal pancreas.

In the present case, although slightly and diffusely enlarged, the pancreas was remarkably normal in contour and in position. A main duct was not distinguished either grossly or microscopically. The small ducts illustrated in Figures 2 and 3 were not parallel with the long axis of the pancreas but formed angles of at least 45° with it. This angle is evident in the reconstruction, in which the vertical axis of the illustration corresponds with the long axis of the pancreas (Fig. 5). Because of their number, size, and position it is thought that these ducts were large branches of the main duct which was no longer present. Although glandular tissue was lacking in much of the pancreas, when present the acini were normally formed and differentiated and were often situated at the periphery of the organ (Fig. 1). The

number of the islets of Langerhans was definitely less than is normal, but even when completely isolated in fibrous stroma the configuration was normal although the cells individually were often shrunken and atrophic (Fig. 4).

As in the kidneys and livers previously reported,[23-25] the normal gross structure of the pancreas implies that the early development of the organ was normal and that differentiation of the components continued normally for a considerable time. It is very likely that the main duct was originally present, but disappeared, probably as the result of segmentation and resorption, following the proliferation of many of its branches. These branches persisted as the numerous, small, irregular ducts, in turn with the distorted outpocketings or branches which are illustrated. It may also be assumed that normal proliferation and differentiation of the glandular tissue occurred simultaneously with segmentation and resorption of the main duct. The process of segmentation and resorption did not stop with the main duct but also involved the smaller ducts and their branches. As a result the glandular tissue became isolated and much of it atrophied and disappeared. Although isolated, some of the islets of Langerhans also persisted. Simultaneously, the degenerating epithelial elements were replaced by proliferating fibrous tissue so gradually that the gross structure of the pancreas remained normal.

If this recapitulation is correct, then the sequence of anatomic changes in the epithelial structures of the pancreas is identical with that which has been postulated for polycystic disease of the kidney and liver. Persistence of many of these isolated segments as gradually enlarging cysts can explain the lesions of polycystic disease in later life. It may be concluded, therefore, that the changes described in the pancreas of the present case are consistent with the theory that in polycystic disease the fundamental defect is segmentation of epithelial tubules and ducts after their formation in accordance with the normal architecture of the organ.

Although the process of segmentation and resorption of the ducts appears to be similar to that which occurs in the polycystic kidney and liver, the greater reduction in the glandular tissue and the much greater amount of fibrous stroma in the pancreas require further comment. It may be argued that the amount of epithelium in the anlagen was deficient from the beginning and that the extensive zones of fibrosis merely represent a condensation of fetal mesenchyma in those areas normally occupied by glandular tissue. This hypothesis seems unlikely, however, since, in contrast to the kidney, in the pancreas both the

glandular tissue and ducts are derived from the same anlage and there appears to be no deficiency of the smaller ducts in the present case. Furthermore, if the epithelial anlagen were actually deficient, the grossly normal development which was found in the pancreas of the present case hardly could have occurred. It may be argued that chronic inflammation of the pancreas, associated with contraction of fibrous tissue, may have constricted many of the pancreatic ducts and led to segmentation and resorption. This hypothesis is supported by the fact that chronic inflammatory exudate has often been reported as extensive. Such a process, however, if actually significant, would almost certainly lead to distortion of the gross structure of the pancreas. The signs of inflammation in the present case were minimal, and it has been previously emphasized by us that inflammation is frequently inconspicuous in polycystic disease of other organs. Consequently, although inflammation may contribute to the segmentation and resorption of epithelial elements in certain cases, it does not appear to be a primary factor in the pathogenesis of polycystic disease.

SUMMARY AND CONCLUSIONS

1. The polycystic lesions of the pancreas in an infant were studied by the usual methods and by a three-dimensional model which is illustrated.

2. The characteristic progressive distortion, cystic dilatation, and segmentation of the ducts are thought to have occurred simultaneously with replacement fibrosis and did not prevent the normal gross development of the pancreas.

3. It is believed that the changes described correspond with those postulated for the polycystic lesions of the kidney and liver, and indicate that in polycystic disease epithelial tubules and ducts are formed in accordance with the normal architectural pattern of the organ but then become distorted and segmented. Instead of complete resorption of these segments, as occurs in the normal degeneration of the mesonephros and in early generations of tubules of the metanephros and liver, many of them persist to form isolated cysts.

REFERENCES

1. Andersen, D. H. Cystic fibrosis of the pancreas and its relation to celiac disease. A clinical and pathologic study. *Am. J. Dis. Child.*, 1938, *56*, 344–399.

2. Andersen, D. H. Cystic fibrosis of the pancreas, vitamin A deficiency, and bronchiectasis. *J. Pediat.*, 1939, *15*, 763–771.

3. Rauch, S., Litvak, A. M., and Steiner, M. Congenital familial steatorrhea with fibromatosis of the pancreas and bronchiolectasis. *J. Pediat.*, 1939, *14*, 462–490.

4. Oppenheimer, E. H. Congenital atresia of the pancreatic duct with cystic fibrosis of the pancreas. *Arch. Path.*, 1940, 29, 790–795.

5. Robbin, L., and Bernhard, W. A. Cystic fibrosis of the pancreas. Report of a case, with clinical and pathologic observations. *Am. J. Dis. Child.*, 1942, 63, 530–540.

6. Wolman, I. J. Cystic fibrosis of the pancreas. *Am. J. M. Sc.*, 1942, 203, 900–906.

7. Daniel, W. A., Jr. Fibrocystic disease of the pancreas. Report of eight cases. *Am. J. Dis. Child.*, 1942, 64, 33–42.

8. Snelling, C. E., and Erb, I. H. Cystic fibrosis of the pancreas. *Arch. Dis. Childhood*, 1942, 17, 220–226.

9. Kennedy, R. L. J., and Baggenstoss, A. H. Fibrocystic disease of the pancreas. *Proc. Staff Meet., Mayo Clin.*, 1943, 18, 487–493.

10. Menten, M. L., and Middleton, T. O. Cystic fibrosis of the pancreas. Report of 18 proved cases. *Am. J. Dis. Child.*, 1944, 67, 355–359.

11. Sears, W. G. Congenital cystic disease of the kidneys, liver, and pancreas. *Guy's Hosp. Rep.*, 1926, 76, 31–52.

12. Teuscher, M. Über die kongenitale Cystenleber mit Cystennieren und Cystenpankreas. *Beitr. z. path. Anat. u. z. allg. Path.*, 1926, 75, 459–485.

13. Bartoli, O. Contributo clinico ed istologico allo studio della patogenesi delle cisti del pancreas. *Arch. ital. di chir.*, 1929, 23, 385–401.

14. Rümler, E. Die polycystische Entwicklungsstörung im Pankreas, zugleich ein Beitrag zur Frage der Cystenleber und der Cystennieren. *Virchows Arch. f. path. Anat.*, 1934, 292, 151–165.

15. Pazzagli, R. Sulla patogenesi e la sintomatologia delle cisti del pancreas. *Rassegna internaz. di clin. e terap.*, 1935, 16, 886–900.

16. Albrecht. Ueber Hamartome. *Verhandl. d. deutsch. path. Gesellsch.*, 1904, 7, 153–157.

17. Kampmeier, O. F. Über das Schicksal der erstgeformten Harnkanälchen der bleibenden Niere beim Menschen. *Arch. f. Anat. u. Physiol., anat. Abteil.*, 1919, 204–226.

18. Kampmeier, O. F. A hitherto unrecognized mode of origin of congenital renal cysts. *Surg., Gynec. and Obst.*, 1923, 36, 208–216.

19. Kampmeier, O. F. Weitere Studien über die Entwicklungsgeschichte der bleibenden Niere beim Menschen. *Ztschr. f. Anat. u. Entwcklngsgesch.*, 1924, 73, 459–500.

20. Kampmeier, O. F. The metanephros or so-called permanent kidney in part provisional and vestigial. *Anat. Rec.*, 1926, 33, 115–120.

21. McKenna, C. M., and Kampmeier, O. F. A consideration of the development of polycystic kidney. *Tr. Am. A. Genito-Urin. Surgeons*, 1933, 26, 377–383.

22. McKenna, C. M., and Kampmeier, O. F. A consideration of the development of polycystic kidney. *J. Urol.*, 1934, 32, 37–43.

23. Norris, R. F., and Herman, L. Observations on the anatomic basis of congenital polycystic kidneys. *Tr. Am. A. Genito-Urin. Surgeons*, 1938, 31, 41–57.

24. Norris, R. F., and Herman, L. The pathogenesis of polycystic kidneys: Reconstruction of cystic elements in four cases. *J. Urol.*, 1941, 46, 147–176.

25. Norris, R. F., and Tyson, R. M. The pathogenesis of polycystic livers. Reconstructions of cystic elements in two cases. *Am. J. Path.*, 1947, 23, 201–215.

26. Lewis, F. T. Development of the Pancreas. In: Keibel, F., and Mall, F. P. (eds.) Manual of Human Embryology. J. B. Lippincott Co., Philadelphia, 1912, 2, 429–445.

[Illustrations follow]

DESCRIPTION OF PLATES

PLATE 81

FIG. 1. The pancreatic acini are normally formed, but the small ducts are distorted and cystic. Hematoxylin and eosin stain. $\times$ 105.

FIG. 2. A medium-sized duct is distorted and is completely surrounded by dense fibrous stroma. At the left, the duct-like structures are in reality small cysts which may be pinched-off branches of the larger duct. Hematoxylin and eosin stain. $\times$ 180.
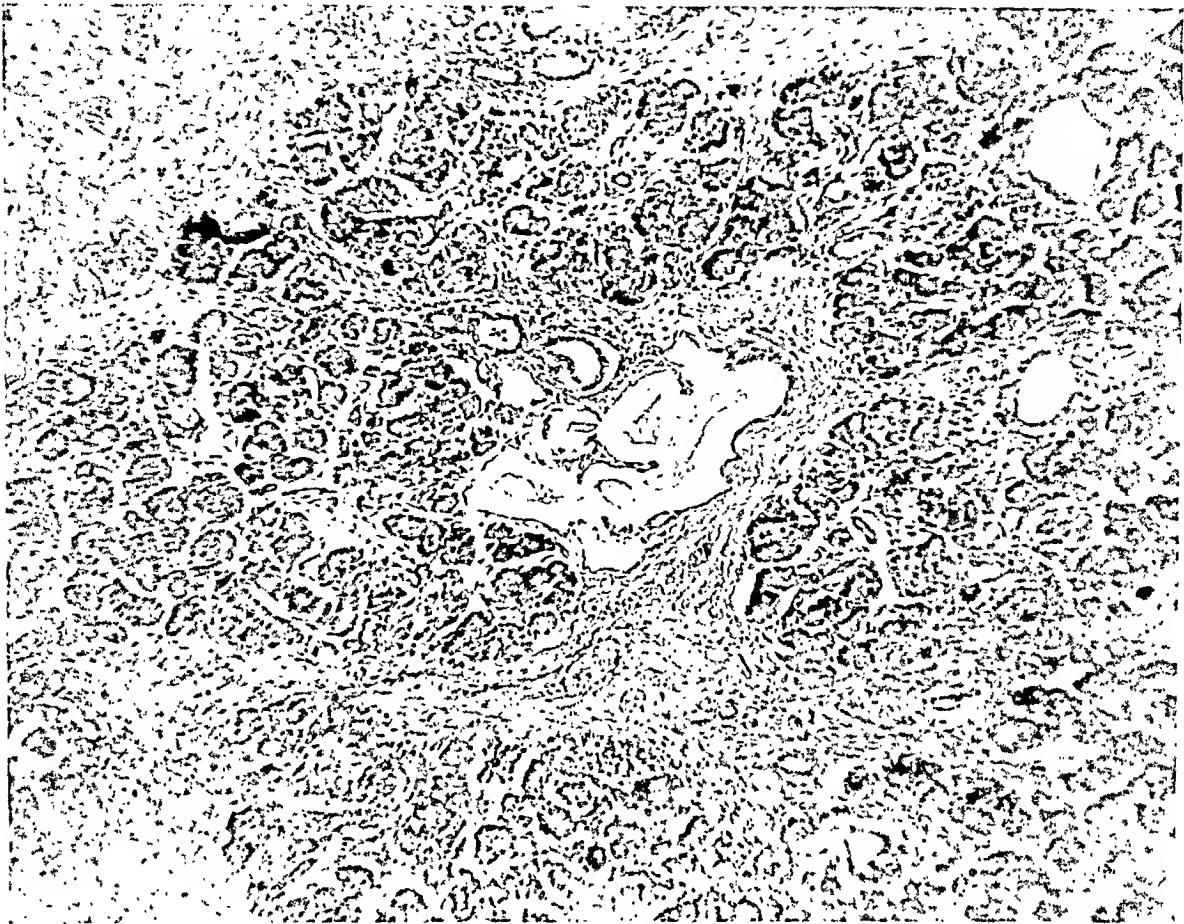
PLATE 82

FIG. 3. In large areas, the pancreatic ducts and their branches are surrounded only by fibrous stroma. Hematoxylin and eosin stain. × 50.

FIG. 4. In the center of the field, three islets of Langerhans embedded in fibrous stroma are normally formed, but the epithelial cells are individually shrunken and atrophic. Hematoxylin and eosin stain. × 105.
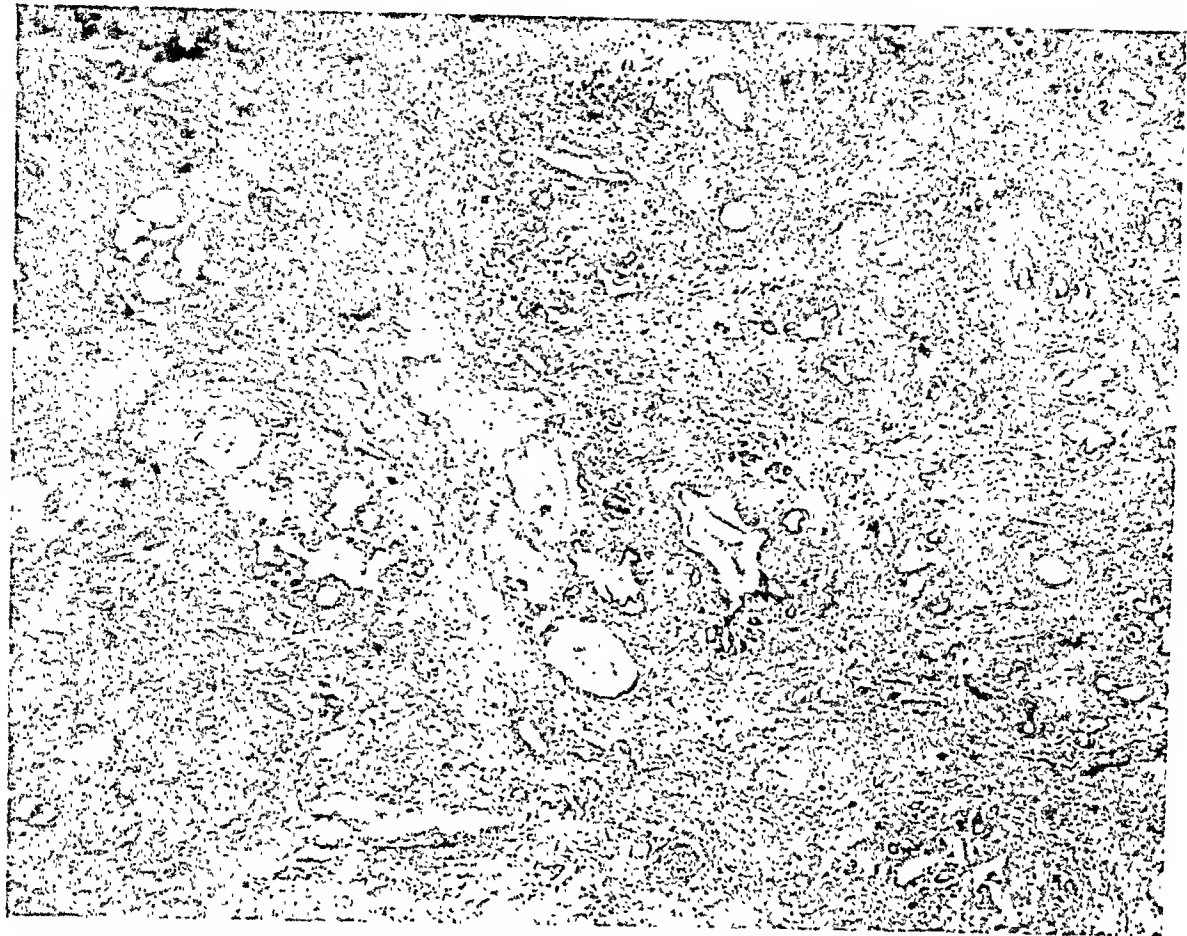
PLATE 83

FIG. 5. The model of one of the larger pancreatic ducts shows the marked irregularity and focal dilatation. There are numerous branches, some pointed and others bulbous. To the left and above the main duct are several isolated, undilated cysts which may be pinched-off segments of some of the branches. The vertical extent of the reconstruction in the pancreas was 0.68 cm.

5

Noris and Tyson

Pathogenesis of Polycystic Pancreas

ECTOPIC SMOOTH MUSCLE IN THE HUMAN GASTRIC MUCOSA *

RAFFAELE LATTES, M.D.

(From the Laboratory of Surgical Pathology of the Surgical Department of the College of Physicians and Surgeons, Columbia University, New York 32, N.Y.)

In the course of routine and methodical histological study of large numbers of surgically removed stomachs, it became apparent that in a significant fraction of these there were definite abnormalities in the nonepithelial component of the mucous membrane, consisting of the presence of conspicuous bundles of smooth muscle in the lamina propria of the gastric mucosa, extending between its glands and occasionally compressing and distorting them. The accompanying photomicrographs show a few examples of the abnormalities referred to. The bundles of smooth muscle may be more or less conspicuous; however, in this study only those cases are included in which the mucosal architecture was definitely abnormal due to the presence of the ectopic muscle.

In some of our cases, formation of a new muscular membrane appeared to have occurred about midway between the muscularis mucosae and the free mucosal surface (Fig. 2). The gastric mucosa was thus divided into two zones: a more superficial one extending down approximately to the pit-neck junction; and a deeper one including the secretory portion of the glands. In other cases, there was a sort of plexiform arrangement of these abnormal muscular bundles, and the gastric glands enclosed in this meshwork could be mistaken for Brunner's glands because of their separation into small lobules circumscribed by the trabeculae of smooth muscle (Fig. 1). In still other cases—the most numerous—the abnormality manifested itself by the presence of scattered muscular bundles between the glands at varying distances from the muscularis mucosae and without a definite pattern. When a sufficient number of sections was taken, it was, in general, possible to establish that at some point there was a connection between the normal muscularis mucosae and the ectopic muscular bundles (Fig. 3).

In some stomachs the muscularis mucosae was dissociated into distinct bundles by the formation of lymphoid collections between them. These cases are not included in the present study.

For several years, it has been a routine practice in our laboratory to take sections of surgically resected stomachs following a standard pattern. By this method it is easy to orient the microscopical preparations in relation to the different regions of the organ. This practice made it possible to establish that, in the majority of the specimens

examined, the bundles of ectopic smooth muscle were situated in the antrum and in the pyloric canal, especially in those portions of the anterior and posterior gastric walls adjacent to the lesser curvature. It also has been my definite impression that these bundles of smooth muscle tended to radiate from the lesser curvature toward both gastric walls, while only exceptionally was their direction parallel to the axis of the lesser curvature.

Little can be found in the medical literature regarding the abnormality which is the subject of this paper. Normal histology teaches that in the mucous membrane of adult stomachs there may be found some individual smooth muscle fibers in the lamina propria, running between the glands and parallel to them. This seems to have little relationship to the findings that are studied here. More interesting is the fact that in the human embryo (Möllendorff,[1] page 113) the smooth muscle of the gastric mucosa begins to differentiate from the stroma of the lamina propria about the 15th week and, in these early stages, shows a very disorderly arrangement throughout the mucous membrane without the formation of a well differentiated muscularis mucosae. Of some interest are other facts from comparative anatomy, *i.e.*, that in some vertebrates, such as birds, reptiles, crocodiles, there is either no well differentiated muscularis mucosae and only irregularly scattered smooth muscle fibers between the glands, or, as in the glandular stomach of birds, there may be a duplication of the muscularis mucosae to form two parallel membranes between which there are portions of the gastric glands (Möllendorff[1]). In the series of human stomachs studied, there were specimens in which the arrangement of the smooth muscle in the mucosa suggested both of these types.

Only a few of the writers who have studied gastric lesions have made mention of these conspicuous bundles of smooth muscle. In the treatise of Henke and Lubarsch, Konjetzny[2] and Hauser[2] described the same abnormality that we are studying here, but interpreted it as a "neoformation" of smooth muscle and connective tissue as a part of a scarring process. Robertson,[3] in 1939, described a "hyperplasia of [the gastric] glands and disorganization of [the] muscularis mucosae" which, from his illustrations, appears to be the same condition here described. He considered it a result of the healing of superficial ulcers. Guiss and Stewart,[4] in their paper on chronic gastritis (1943), stated that "in the pyloric region smooth muscle extensions of the muscularis mucosae are normally found between the pyloric glands." This is "not a constant finding and as a criterion for a microscopic diagnosis of chronic atrophic gastritis it is probably not reliable."

Gitlitz and Colp,[5] in an excellent paper on "Gastric Histology and Subtotal Gastrectomy" (1943), described a proliferation of collageno-muscular tissue in the mucosa, usually accompanied by atrophy and disappearance of glandular elements. From their careful description, it is obvious that their findings are identical with mine. Their interpretation seems to be that these changes are the result of hyper-regeneration in the process of repair of mucosal ulcers.

As we have seen, a few authors have observed the presence of abnormal smooth muscle bundles in the human gastric mucosa. Some have regarded it as a sign of hyper-regeneration of the muscularis mucosae occurring as a part of the process of chronic gastritis or as a replacement of atrophic glands. Others have believed that it probably has no pathological significance and that it may represent a sort of individual variation within the limits of normal.

It is difficult to agree with the theory of regeneration of smooth muscle. Save a few and well known exceptions (pregnant uterus, new vessels in canalized thrombi), the normal smooth muscle is not known to proliferate or regenerate to any extent. In the stomach, I have never seen any sign of proliferation of smooth muscle fibers in association with erosions or ulcers of the gastric mucosa. These injuries of the smooth muscle have healed by fibrous tissue scars.

TABLE I

Partition of 100 Unselected, Surgically Removed Stomachs as to Diagnosis

Gastric ulcers	18
Duodenal ulcers	57
Jejunal ulcers	5
Carcinomas	23
Lymphosarcomas	2
Total	105
Less 5 cases of multiple gastroduodenal ulcers	5
	100

After having observed this abnormality in a considerable number of stomachs, an attempt was made to analyze our cases with the purpose of ascertaining a possible relationship between the histological findings and the clinical and radiological symptoms. With this in mind, I reviewed the anatomo-pathological, radiological, and clinical pictures of an unselected group, comprising a series of 100 examples of partial gastrectomy performed in the Surgical Department of the Presbyterian Hospital during a period of about 2 years. Table I presents these cases subdivided according to their respective diagnoses. In this group, 56 cases showed bundles of aberrant smooth muscle in the lamina propria. The distribution of this abnormality in this series is analyzed in Table II. When the respective percentages are analyzed, it becomes obvious that there is no significant correlation of this finding with any of the pathological conditions included in this series.

Table III is an attempt to compare the frequency of those radiological, clinical, and anatomo-pathological features that were considered of greatest significance in the cases having supernumerary smooth muscle in the gastric mucosa with their frequency in the cases in which this finding was not present. The incidence of the radiological features

TABLE II

Relation of Ectopic Musculature to Diagnosis of 100 Resected Stomachs

Fifty-six examples of ectopic smooth muscle occurred with:
34 duodenal ulcers (59% of all duodenal ulcers)
8 gastric ulcers (44% of all gastric ulcers)
12 carcinomas (52% of all carcinomas)
2 marginal (jejunal) ulcers (40% of all marginal ulcers)

under consideration was not significantly different in the two groups. The average duration of the symptoms was somewhat higher in the first group. The only significant difference was that in the cases with bundles of supernumerary smooth muscle in the gastric mucosa the incidence of pyloric hypertrophy was more than twice that found in the other cases.*

TABLE III

Comparative Incidence of Other Findings in Stomachs With and Without Ectopic Smooth Muscle

	Ectopic smooth muscle	
	Present	Absent
Delayed emptying of stomach (roentgenologically)	61%	50%
Exaggerated mucosal folds of antrum (roentgenologically)	50%	45%
Hypertrophy of pylorus	59%	27%
Average duration of symptoms	6.9 yrs.	4.4 yrs.

In conclusion, in a significant number of surgically resected stomachs, abnormal smooth muscle bundles can be found in the lamina propria. These can be compared with those in some animal species (reptiles) and perhaps in the human embryo. No mention of this muscle is found in any treatise of human histology and only rarely can it be found in papers dealing with chronic gastritis and with the pathology of the stomach in general. To my knowledge no satisfactory explanation has been offered.

Logically, it would seem that some significance must be attached to this feature because of its relative frequency. In this series, the only significant facts related to the presence of this muscle were a frequent

---

* The pyloric muscle was considered hypertrophied when its thickness, measured in the opened specimen, was 5 mm. or more.

hypertrophy of the pylorus and a longer duration of the symptoms of gastric disease.

As a tentative explanation, I believe that these findings may represent hypertrophy and perhaps hyperplasia of the normal rudimentary muscle of the lamina propria of the human gastric mucosa, perhaps directed towards increasing the formation of the longitudinal mucosal folds grossly described and studied by Forssell [6] and by him interpreted as directed toward facilitating the emptying of the fluid or semi-fluid contents of the stomach.

SUMMARY

In a considerable number of surgically resected stomachs, the mucosa of the antral pyloric region has shown an abnormality consisting of conspicuous bundles of smooth muscle extending between the glands and appearing to radiate from the lesser curvature toward both anterior and posterior gastric walls. An attempt is made to interpret this abnormality on the basis of the anatomo-pathological and clinical findings as hypertrophy and possibly hyperplasia, perhaps directed toward increasing the longitudinal mucosal folds.

REFERENCES

1. Möllendorff, W. v. Handbuch der mikroskopischen Anatomie des Menschen. J. Springer, Berlin, 1932, 5, Pt. 2.
2. Konjetzny, G. E. Die Entzündungen des Magens. In: Henke, F., and Lubarsch, O. Handbuch der speziellen pathologischen Anatomie und Histologie. J. Springer, Berlin, 1928, 4, Pt. 2, 875. Hauser, G. Die peptischen Schädigungen des Magens, des Duodenums und der Speiseröhre und das peptische postoperative Jejunalgeschwür. *Ibid.*, 1926, 4, Pt. 1, 436.
3. Robertson, H. E. Ulcerative gastritis and residual lesions. *J. A. M. A.*, 1939, 112, 22–27.
4. Guiss, L. W., and Stewart, F. W. Chronic atrophic gastritis and cancer of the stomach. *Arch. Surg.*, 1943, 46, 823–843.
5. Gitlitz, A. J., and Colp, R. Gastric histology and subtotal gastrectomy. *Ann. Surg.*, 1943, 118, 523–550.
6. Forssell, G. The rôle of the autonomous movements of the gastrointestinal mucous membrane in digestion. *Am. J. Roentgenol.*, 1939, 41, 145–165.

[*Illustrations follow*]

DESCRIPTION OF PLATES

PLATE 84

FIG. 1. Ectopic smooth muscle arranged in plexiform fashion, circumscribing groups of gastric antral glands.

FIG. 2. Ectopic bundles of smooth muscle which appear to form a second muscularis mucosae at the level of the pit-neck junction.
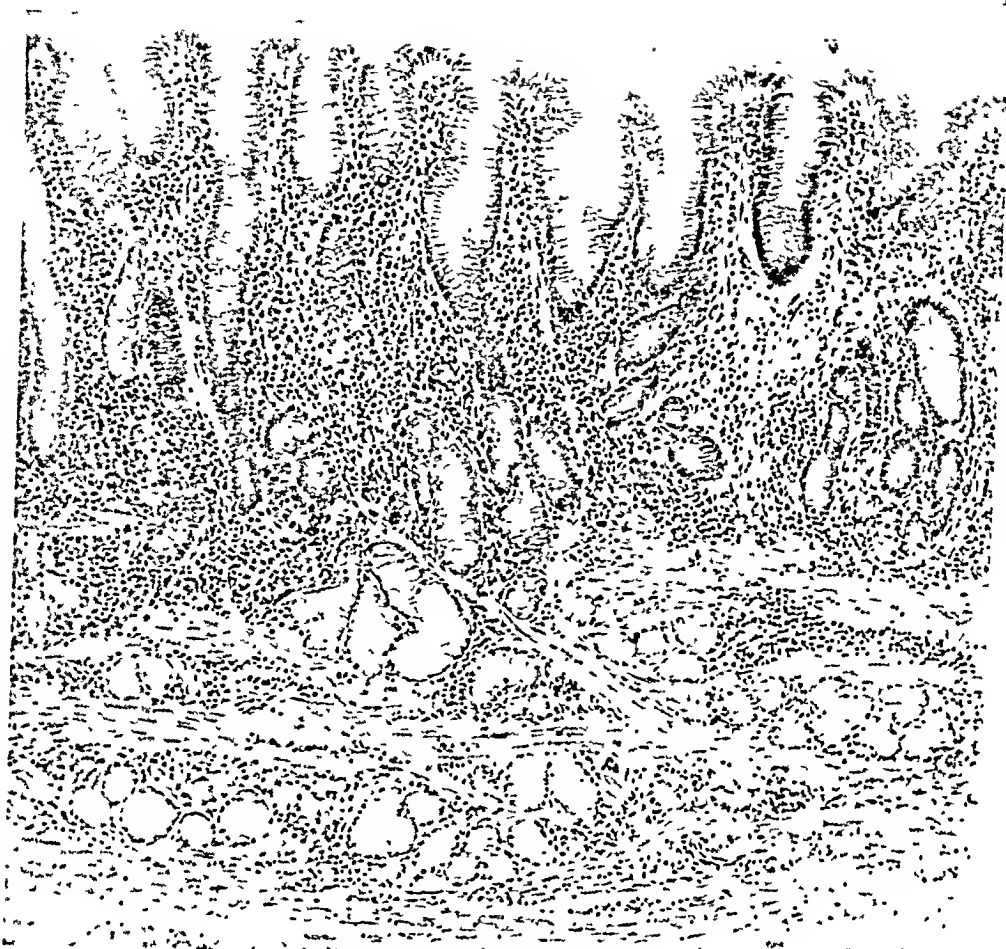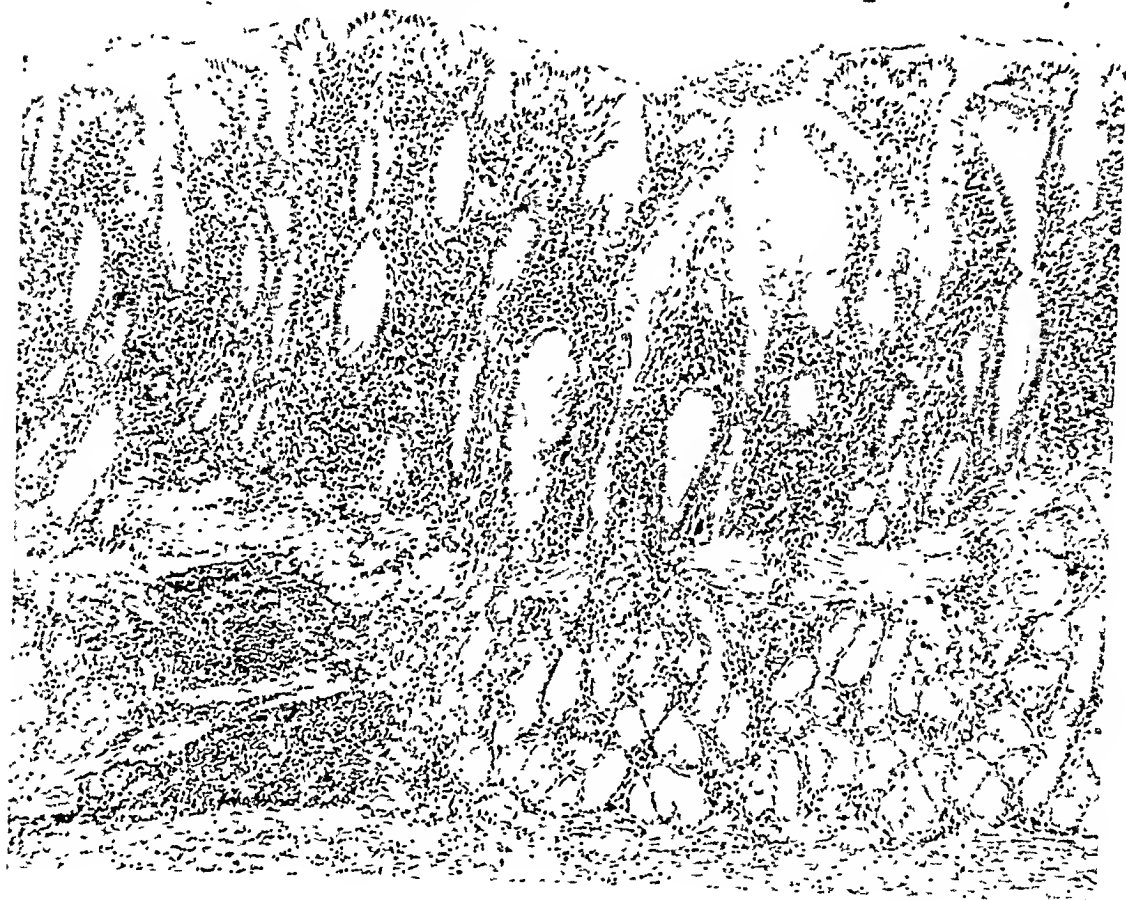
Lattes

Smooth Muscle in Gastric Mucosa

PLATE 85

FIG. 3. A conspicuous bundle of smooth muscle arising obliquely from the muscularis mucosae.

3

Lattes

Smooth Muscle in Gastric Mucosa

PLATE 86

FIG. 4. Group of gastric antral glands completely surrounded by abundant ectopic smooth muscle.

4

Lattes

Smooth Muscle in Gastric Mucosa

THE RENAL ARTERIOLAR CHANGES IN THE ANURIC CRUSH SYNDROME *

N. GOORMAGHTIGH

(*From the Department of Pathology, University of Ghent, Ghent, Belgium*)

The most striking changes reported hitherto in the kidneys of patients with the anuric crush syndrome concern the ascending limb of the loop of Henle and the second convoluted tubule,[1,2] and have been extensively discussed by Bywaters and Dible.[2] They account for the loss of selective reabsorption observed in cases of fatal crush syndrome but they fail to give a satisfactory explanation of the marked oliguria and the gradual increase of blood pressure.

In a recent paper [3] I have drawn attention to the renal *arteriolar* changes in crush syndrome. Their elucidation requires familiarity with the existence of a juxta-glomerular apparatus and, possibly, acceptance of glandular function for certain cells in the media of the renal arterioles.[4-8]

The purpose of this paper is to analyze these changes in detail and to attempt a precise definition of the renal lesion which may follow the release of toxic substances by crushing injuries to the muscles of the limbs.

MATERIALS AND METHOD

I have investigated the histologic changes in four kidneys from patients with crush wounds (cases A-1, A-3, A-4, A-5). The kidneys were swollen, edematous, and grayish pink. They were fixed in a 10 per cent solution of neutral formalin and had been submitted to me by pathologists of the Royal Canadian Army Medical Corps stationed in Belgium. The victims were young enlisted men in the Canadian Army who had died 4 to 9 days after crushing injuries and after the development of marked oliguria and even anuria (case A-4). Unfortunately, no detailed clinical or post-mortem report was available. However, in cases A-3 and A-5 the blood pressure had been measured once on the fourth day: in the former the systolic blood pressure was 150 mm. of

Hg and the diastolic was 70 mm. of Hg; in the latter the systolic blood pressure was 155 mm. of Hg and the diastolic was 75 mm. of Hg.

Sections from paraffin blocks were stained by Masson's trichrome iron-hematoxylin, fuchsin, and aniline blue.

A case of "traumatic uremia" observed by me during the war of 1914–18 is also included. The clinical data in this instance are summarized as follows: V. d. B., 23 years old; wounded in the shoulder by a shell splinter; left axillary artery torn with formation of a large hematoma, accompanied by hemorrhagic shock. Ligature of the left axillary artery and vein was performed and a blood transfusion was given. Incipient ischemic gangrene of the left arm appeared on the fifth day. Oliguria became notable (urinary output: maximum, 285 cc.; none on the sixth day). Urine: clear, albumin in small amount; uremic symptoms included vomiting. Blood pressure: 60/50 mm. of Hg on admission; 130/70 on the second and third days; 145/70 on the fourth; 140/60 on the fifth; 170/60 on the sixth day. Hemodilution was shown in the erythrocyte counts, which fell from 3,900,000, 5 hours after trauma, to 2,100,000 red blood cells per mm. shortly before death in uremic coma on the sixth day. Kidneys were fixed in Bouin's and Flemming's fluids. This case is designated as no. 76.

Microscopic sections in a sixth case were kindly put at my disposal (A-12-B) by Major McManus, Royal Canadian Army Medical Corps. These were from a case of traumatic uremia in a soldier, 40 years of age, following hemorrhage, extensive infection of muscle wounds, and blood transfusion.

OBSERVATIONS

The Glomeruli

As in previous papers, I have adopted Zimmermann's description of the structure of the glomerular tuft, according to which the loops of the latter are supported by a mesangium.[9] This formation consists of thin, often bifurcated lamellae, the ground substance of which comes out clearly with aniline blue (Fig. 1). The mesangium cells contain a round or oblong nucleus with densely packed chromatin. Their cytoplasm is scarce but shows slender expansions (at a magnification of 700 ×) which, stained with acid fuchsin, contrast sharply with the blue ground substance and give to the mesangium a heterogeneous aspect. Some of these expansions are in the free wall of the loops which constitutes the filtration membrane. This membrane is, in fact, formed by the coalescence of two basement membranes: that of the epithelial cells (Deckzellen) and that of the endothelial lining of the loops (Zimmermann [9]).

In cases of crush wound or traumatic uremia, the size of the glomerular tufts and the diameter of their loops varied. In some specimens the tufts were retracted (cases A-3 and A-4); in others, usually relaxed (cases A-1 and A-5). These variations did not depend on congestion or depletion of the loops. Most of the tufts were almost bloodless but they contained remnants of shrunken, hemolyzed erythrocytes (Figs. 1 and 2) which contrasted with the few preserved erythrocytes (stained black with iron hematoxylin) occasionally present in the loops and with the abundant erythrocytes observed in the intertubular capillaries. Other changes were noticed in the tuft. Often, the endothelial cells of the loops enlarged and resembled endothelial leukocytes (Fig. 1). Polymorphonuclear leukocytes were retained. Clusters of thrombocytes were found occasionally; hyaline thrombi, exceptionally (cases A-1 and A-4). When the tufts were relaxed (maximal relaxation found in case A-1) the loops were filled with vacuolated blood-plasma, faintly stained with aniline blue. In these cases, also, the infundibulum, from which originate the loops of the tuft (schema of Vimtrup), was large and distended with blood-plasma. Groups of normally stained erythrocytes were sometimes found to be floating in this fluid (case A-1). The wall of the infundibulum sometimes showed an increased number of spindle-shaped cells separated by fine collagen fibrils.

I have not noticed any striking changes in the mesangium except in cases A-1 and A-5 (Fig. 1). In many glomeruli of these cases the heterogenous structure of the mesangium had vanished and the ground substance had increased. Also pyknosis of the nuclei occurred. In apparently unchanged mesangia (cases A-3, A-4, 76, and A-12-B) hypertrophic or lobulated nuclei were discovered after an exhaustive examination.

The epithelial lining of the tuft offered no characteristic changes except in case A-1 where it was atrophic and degenerated in places (Fig. 1). The epithelial layer of Bowman's capsule rarely reacted and, if so, not very markedly. Apparently this occurred only when fibrinoid material, stained bright red with acid fuchsin, was deposited in the glomerular space—a very rare occurrence.

The distention of the glomerular capsule was caused either by the accumulation in the glomerular space of albuminous, grossly globoid (after fixation) material, or by the enlargement of the tuft itself (case A-1). It entailed the incorporation of the hypertrophic epithelial lining of the neck of the proximal convoluted tubule in the distended glomerular capsule.

When the tufts were excessively distended and when the mesangium was in regression, the so-called catarrh of the proximal convoluted

tubule was striking (case A-1). In its lumen the same kind of globoid material was found as in the glomeruli. On the other hand, this material was scanty and granular when the tuft was retracted and the mesangium was intact.

Hyalinized glomeruli were not more numerous than in normal persons of the same age.

Fat embolism of the tufts of moderate degree was an incidental finding.

The Post-Glomerular Arterioles

As a rule the lumina of the post-glomerular arterioles were wide and empty. Occasionally they contained a few hemolyzed erythrocytes close to the glomeruli and, exceptionally, normally stained erythrocytes. The media was unchanged and contained a small number of nongranular afibrillar cells. The presence of minute patches of subendothelial exudate was a constant feature (cases A-3, A-4, A-5, 76, and A-12-B, and especially A-1 and A-5). The lymphatics which run along the efferent arterioles were filled with mononuclear cells.

The Pre-Glomerular Arterioles

The pre-glomerular arterioles (juxta-glomerular apparatus included) show the most conspicuous changes. These come under two headings: (a) presence of numerous granular afibrillar cells in the media (Fig. 4), and (b) the presence of subendothelial patches of bluish stained transudate.

Before describing the granular afibrillar change of the media it must be emphasized that I have never found granular afibrillar cells in the arterioles of human kidneys unless there was hypertensive disease or a condition of anemia or serious liver damage. Graef [10] also found that in man the afibrillar cells of the renal arterioles normally are devoid of granules.

In crush wounds or traumatic uremia the intensity of the granular change of the smooth muscle cells varies. In case A-4 this transformation was confined chiefly to the juxta-glomerular apparatus (Fig. 6). In case 76 the muscular cells of the arterioles connected with superficial glomeruli had also become granular (Fig. 4). In case A-1, in which there was evidence of incipient damage to the tuft, the granular afibrillar transformation was maximal; it involved the afferent arteriole along its entire course and even the interlobular pre-arteriole. This granular reaction varied also qualitatively. In case A-4, in which the tufts were intact and retracted, the granules were very small and densely packed as in the kidney of the rabbit. In all other specimens they were coarse (Figs. 5 and 6) and sometimes irregular. The various types of granules

are demonstrated in Figure 1. Some stained bright red with acid fuchsin; others were black with iron hematoxylin. The fixation and staining technics may, to a certain extent, account for these variable staining properties; for the present it is advisable not to lay too much stress upon them. It must be emphasized, however, that the structure of the granular afibrillar cells varied considerably (Fig. 4). It was easy to follow the various stages of glandular activity which led ultimately to the dissolution of the granules and intense vacuolation. The latter process was very marked in cases A-1 and A-5 (Fig. 3): in many juxta-glomerular apparatuses the vacuolated cells predominated while the granular cells became rare. In the former, and to a lesser extent in case A-5, groups of granular smooth muscle cells had degenerated and shrunk. There were extracellular and intracellular lipoid drops in the juxta-glomerular apparatus of case 76 (Flemming's fixation). In cases A-3, A-4, A-12-B, and 76 the fibrillar smooth muscle cells formed the bulk of the media (Fig. 4); their myofibrils were conspicuous. They lay close to the endothelium while the afibrillar cells were in a marginal position. In the juxta-glomerular apparatus, however, the latter came close to the endothelium (Fig. 5). In case A-1 and sometimes in case A-5 most of the media cells were afibrillar; the lumen of the vas afferens was large and filled with pale-staining plasma. Some preparations from case A-1 suggested that the distention of the lumen results from a circulatory disturbance in the glomerular tuft.

In the pre-glomerular arterioles there were patches of subendothelial, finely vacuolated transudate; they stained blue with the Masson stain and must be considered as incipient arteriolosclerosis (Figs. 2 and 3). In cases A-1, A-4, 76, and A-12-B they were small and were found in the juxta-glomerular apparatus. In cases A-2 and A-5 they involved large segments of arterioles. Granular afibrillar cells were always found close to or in the patches. Often these cells are signs of incipient regression or excessive vacuolation (Figs. 2 and 3).

The Intertubular Capillaries

In contrast to the glomeruli, the intertubular capillaries were filled with intact erythrocytes. However, in some limited areas and especially around the glomeruli (cases A-3, A-4, A-12-B, and 76) a few capillaries were relaxed and devoid of erythrocytes. They contained coagulated plasma and occasionally monocytes, polymorphonuclear leukocytes, and blood-platelets in variable amount. In cases A-1 and A-5 these areas were larger; the basement membrane of the capillaries was thickened and collagenous fibrils were deposited between them, apparently without histiocytes (Fig. 7).

Except in the superficial layer of the cortex, interstitial edema was considerable and in some places was accompanied by histiocytic and fibroblastic proliferation. The latter was conspicuous in the boundary zone where the accumulation of pigment casts, the tubular damage, and the extrusion of casts were most marked (Bywaters and Dible [2]).

Interlobular Veins

The lesions of the veins which have been reported briefly in a previous paper [3] deserve special study, the result of which I intend to publish later.

DISCUSSION

The changes described in this paper appear to be related to conditions prevailing shortly before death. The extreme glomerular ischemia, observed in my preparations, cannot have been of long duration because of the very slight morphologic damage to the tuft in all cases except two (cases A-1 and A-5). However, the retention of shrunken remnants of erythrocytes, the occasional presence of a few intact erythrocytes, the accumulation of polymorphonuclear leukocytes and blood-platelets, and, finally, the presence of blood-plasma in the loops of the tuft, suggest a sluggish or intermittently interrupted glomerular circulation for a considerable time before death. In most cases, glomerular filtration probably continued at a reduced rate but the filtrate contained an albuminous coagulum.

In discussing the mechanism which induces glomerular ischemia it must be kept in mind that the arterial blood pressure in patients suffering from crush wounds is either normal or high shortly before death; [11] that the lumen of the pre-glomerular arterioles is distended; that the number of the contractile elements of the arteriolar media has decreased; and that the retraction or relaxation of the tuft does not depend on the degree of vascularization of the tuft. In accordance with an opinion previously expressed,[7] I admit that the tone of the glomerular loops is controlled to some extent by the cells of the mesangium of Zimmermann which are of the same lineage as the muscle cells of the arterioles (de Winiwarter [12]) and are intact in most of my cases. The tuft is not a system of capillaries but an arteriolar segment adapted to the function of filtration.[7,8] I suggest that during the last days of the anuric crush syndrome the glomerular tuft contracts and in some cases ultimately relaxes. The relaxation should occur when the mesangium becomes functionally defective.

I also suggest that the numerical increase of the afibrillar granular cells in the media of the pre-glomerular arterioles is the result of a circulatory disturbance in the tufts, or in the arteriolocapillary junc-

tions further down stream, because a similar retrograde change occurs in the pre-glomerular arterioles of excessively ischemic kidneys. In the latter the cessation of glomerular circulation obviously precedes the obliteration of the afferent arterioles; these remain patent for some time and become blind alleys when the tufts are reduced to hyaline blocks; as the supply of oxygen to the muscular coating of these *culs-de-sac* decreases, there follows the appearance of granular cells all along their course.[7] According to my previous work,[7] confirmed by Dunihue and Candon,[13] these cells are believed to produce vasopressive or pre-vasopressive substance. There are reasons to believe that, normally, this substance acts on the neighboring contractile smooth muscle cells but overflows in the general circulation and causes hypertension when produced in excessive amount. I submit that the intensive glandular transformation of the renal arteriolar wall is one of the main factors responsible for the rise of blood pressure in crush syndrome. This claim is supported by the fact that whenever the afibrillar cells increase considerably in number and give evidence of enhanced glandular activity the renin content of the blood is also increased (abrupt rise of blood pressure after placing the Goldblatt clamp, hemorrhagic shock, eclampsia, fulminating acute glomerulonephritis (cf. Dexter *et al.*[14,15]).

This glandular transformation of the media of the pre-glomerular arterioles could be considered as morphologic evidence of a vasopressive compensatory reaction against a circulatory disturbance further down stream. Its intensity seems to be roughly proportional to the degree of the disturbance. In the Goldblatt experiment as long as the urinary function remains unimpaired the reaction is confined to the juxtaglomerular apparatus; but when, as the result of glomerular and tubular regression, the urinary function is reduced or lost, it extends up stream and involves even the interlobular pre-glomerular arterioles. A similar gradation is seen in the different specimens of crush-wound kidneys examined by me. The most extensive arteriolar changes occur when there is a marked deterioration of the intertubular and glomerular circulation. It is evident that this compensatory mechanism is efficient only when the peripheral circulation is slightly interfered with. In more severe cases no contractile substratum remains for the vasopressive substance to act upon because all of the fibrillar cells have become afibrillar and glandular.

I suggest that the correction of the deficiency of the arteriolo-capillary junctions and capillaries (excessive and prolonged vasoconstriction or excessive paralytic dilatation) by the increased tone of more proximal arteriolar segments can be achieved either by a nervous or by

a musculo-endocrine mechanism.[6] If the latter intervenes predominantly, as in the case of crush-wound syndrome, irreversible changes such as arteriolosclerosis are inevitable, and the chain of events described in the textbooks for this vascular lesion is bound to unfold if the patient lives long enough.

I believe that the notion of the existence of a musculo-endocrine regulation of the arteriolar tone will prove useful in the study of hypertensive diseases and arteriolosclerosis.

The experimental data available so far seem to confirm the early onset of renal vasoconstriction in crush wounds. Keele and Slome[16] observed a fall of the blood pressure and a marked reduction of renal blood flow immediately following the release of complete ischemia of a limb maintained for 4 to 5 hours in anesthetized cats. This reduction was more marked than in animals in which the blood pressure was lowered to the same extent by hemorrhage. In these early stages, however, vasoconstriction seems to involve only the post-glomerular arterioles, for Eggleton, Richardson, Schild, and Winton[17] observed glomerular congestion in the dog during the first 48 hours. The blood casts observed by Duncan and Blalock[18] in similar experiments also point to a condition of stasis in the glomerular tufts in the early stages. As generally accepted, the constriction of the post-glomerular arterioles is one of the normal mechanisms regulating glomerular circulation and filtration (Smith,[19] Richards[19a]), but obviously, when it is excessive, it impairs both glomerular and tubular functions. This seems to happen in the early stages of experimental crush wounds (Eggleton et al.[17]). My observations on post-mortem material suggest that in human patients surviving 4 to 9 days, vasoconstriction extends from the post-glomerular arterioles to the glomerular arterioles to the glomerular tufts. This would explain why pigment casts are eliminated with the urine in the early stages and retained in the kidneys towards the end: in the beginning, the flow of urinary filtrate from congested glomeruli is strong enough to wash away the casts; later on, when the glomeruli remain ischemic for long periods, the flow becomes too weak to do so.

I believe that renal deficiency observed in the crush syndrome is the result of vasoconstriction involving first the post-glomerular arterioles and later the glomerular tufts. Vasoconstriction can be followed by paralytic dilatation (as in cases A-1 and A-5). The gradual rise of the blood pressure seems to be caused by the increased vasopressive endocrine function of the pre-glomerular arteriolar wall following the deterioration of the circulation in the tufts, in the post-glomerular arterioles, and in the intertubular capillaries.

The leakages in the distal segments of the tubules (Dunn et al.,[1]

Bywaters and Dible [2]) probably lower the urinary output still more. In discussing the urinary function, glomeruli and tubules must not be considered independently. The existence of a connection at the level of the macula densa suggests a possible functional relationship between the two parts of the nephron.[7] For the time being I cannot say definitely whether the arteriolar constriction is the result of a direct and early impact of a substance of muscular origin on the arterioles or is an indirect one caused by the altered glomerular filtrate and by the damaged renal tubules so closely connected with the glomerular arterioles. In favor of the first of these two possibilities is the fact that, from the onset, peripheral vasoconstriction has usually been observed in crush-syndrome patients. Moreover, the casts and tubular lesions can be the effect of a primary severe ischemia of the kidney (Scarff and Keele [20]).

The patches of subendothelial transudate in the pre-glomerular and post-glomerular arterioles, present in all cases examined, may have existed prior to the muscular injury or may have been caused by it. If the former interpretation is correct, it would mean that incipient renal arteriosclerosis predisposes to the severe vasoconstrictive effect of ischemic muscular necrosis. But the second interpretation is more plausible because severe hypohemia, by increasing considerably the permeability of the endothelium, induces the formation of patches of transudate stainable with aniline dyes in the Goldblatt experiment.[7] Moreover, in crush-wound kidneys the presence of degenerated granular cells in the transudate indicates that the latter is of recent date. In my recent monograph[7] I have given the reasons in support of the opinion that the vasopressive substance is formed to a great extent within the wall of the renal arterioles as the result of the activation of transuded plasma globulin by the glandular product of the endocrine muscle cells. Braun-Menendez and Page (cited by Houssay and Dexter [21]) considered only the action of renin on plasma globulin in the blood stream. Incipient arteriosclerosis should be considered as a deviation of this intraparietal activation of plasma globulin.

Why are the intertubular capillaries filled with intact erythrocytes in spite of the deficient glomerular circulation? The blood is supplied to them by the subcapsular capillary network and by pre-glomerular Ludwig branches. In respect to the Ludwig branches it may be useful to give more information. In a child, 12 years of age, who died from subacute glomerulonephritis, the vasa afferentia and efferentia of nine deep-seated glomeruli were reconstituted; from the vas afferens of two of them a large Ludwig arteriole branched off and supplied capillaries in the cortex as well as in the medulla. Figure 1 of my paper on renal

ischemia [4] gives an appropriate idea of the frequency of these arterioles in the kidney of the dog.

Though fairly numerous in the deeper zone of the renal cortex, these branches do not seem to supply enough blood to restore the intertubular circulation to normal in cases of crush wound. There are indications of a sluggish circulation in some parts of the intertubular capillary network, especially around the glomeruli (accumulation of monocytes and polymorphonuclear leukocytes, stainable blood-plasma). The slowing down of the blood stream is probably one of the causes of the interstitial edema, the other being leakages in the distal part of the nephron.

The renal vascular changes under discussion are identical with those of cases labeled as "traumatic uremia" in which muscle laceration (*e.g.*, by shell splinters) is accompanied by severe wound infection (as in cases 76 and A-12-B), and also with those of eclamptic anuria [5] and acute glomerulonephritis. In the two latter conditions the existence of arteriolar spasms is generally agreed upon. In other words, the renal lesion of the crush syndrome resembles that in acute glomerulonephritis and the vascular reaction considered in this paper is perhaps allergic. It is interesting to note that in cases of eclampsia and fulminating acute glomerulonephritis in which the increase of the granular, glandular formation of the afibrillar cells is so striking, significant amounts of renin have been detected in the blood (Dexter and Haynes [15]).

The vasculo-nephrotoxic substance released by autolysis of the muscle tissue is probably some early intermediate breakdown product of a large protein molecule formed under strictly anaerobic conditions (Eggleton [22]), which is possibly cytotoxic. This may account for the frequent observation of centrolobular necrosis of the liver in fatal crush wounds (Bywaters and Dible [2]) and in cases where severe aerobic infection of lacerated muscle favors anaerobic autolysis after exhaustion of the oxygen (case 76). In mentioning these hepatic lesions it must be kept in mind that the liver is the probable source of hypertensinogen.

Although I cannot make any conclusive contribution to our knowledge regarding the nature of the substance which interferes with the tonus of the renal arterioles in cases of crush wound, it may be worth while to report the fortuitous observation which follows: In a rabbit undergoing a chronic "Goldblatt experiment," rupture of the psoas muscle accompanied by a large hematoma occurred accidentally; this lesion was followed 48 hours later by a very marked centrolobular hepatic necrosis and incipient circulatory disturbances in the glomeruli

of the kidney. This observation suggests that the nephrotoxic substance is not myohemoglobin (as tentatively proposed by Bywaters and Stead [23]) since this substance does not exist in the skeletal muscles of the rabbit.

A systematic investigation of the effect of anaerobic autolysates of muscle tissue and of the resorption of large hematomata on the endocrine cells of the renal arterioles may open an interesting field for further research.

SUMMARY AND CONCLUSIONS

1. In six cases, evidence was found to indicate that renal deficiency in the "crush syndrome" and "traumatic uremia" is to a great extent the result of vasoconstriction (followed by paralytic vasodilatation), involving first the *post-glomerular* arterioles and later the glomerular tufts.

2. As in the Goldblatt experiment, it is suggested that the gradual increase of the blood pressure observed in these cases may be accounted for by a striking increase of *the afibrillar cells of the media of the* pre-glomerular arterioles, which acquire cytologic features of glandular activity and are considered to participate in the formation of a vasopressive substance.

3. Subendothelial patches of freshly coagulated blood-plasma are constantly found in the pre-glomerular and post-glomerular arterioles. These patches are found in the vicinity of granular afibrillar cells which show signs of incipient regression. It is suggested that this may be evidence of an interaction of the blood-plasma and the hormone produced by the afibrillar cells, within the arteriolar wall.

4. The glandular transformation of the media of the pre-glomerular arterioles follows circulatory disturbances in the glomeruli and more peripheral vessels. It is suggested that this transformation is the morphologic expression of a vasopressive compensatory reaction which, in favorable conditions, can restore the deficiency of the more peripheral circulation.

5. The renal lesions observed in fatal crush wounds or traumatic uremia are identical with those of eclamptic anuria and fulminating glomerulonephritis.

REFERENCES

1. Dunn, J. S., Gillespie, M., and Niven, J. S. F. Renal lesions in two cases of crush syndrome. *Lancet*, 1941, 2, 549–552.
2. Bywaters, E. G. L., and Dible, J. H. The renal lesion in traumatic anuria. *J. Path. & Bact.*, 1942, 54, 111–120.
3. Goormaghtigh, N. Vascular and circulatory changes in renal cortex in the anuric crush syndrome. *Proc. Soc. Exper. Biol. & Med.*, 1945, 59, 303–305.

4. Goormaghtigh, N. Histological changes in the ischemic kidney with special reference to the juxta-glomerular apparatus. *Am. J. Path.*, 1940, 16, 409–416.

5. Goormaghtigh, N. La doctrine de la cellule musculaire, afibrillaire, endocrine en pathologie humaine. 2. Le rein de l'éclampsie puerpérale. *Bull. Acad. roy. de méd. de Belgique*, 1942, 7, s. 6, 194–217.

6. Goormaghtigh, N. La vasomotricité musculaire endocrine. *Bull. Acad. roy. de méd. de Belgique*, 1943, 8, s. 6, 502–508.

7. Goormaghtigh, N. La fonction endocrine des artérioles rénales. R. Fonteyn, Louvain, 1944.

8. Goormaghtigh, N. Facts in favor of an endocrine function of the renal arterioles. *J. Path & Bact.*, 1945, 57, 392–393.

9. Zimmermann, K. W. Über den Bau des Glomerulus der Säugerniere. *Ztschr. f. mikr.-anat. Forsch.*, 1933, 32, 176–278.

10. Graef, I. Medial hypertrophy of the renal arterioles in pregnancy. *Am. J. Path.*, 1943, 19, 121–133.

11. Bywaters, E. G. L. Ischemic muscle necrosis. *J. A. M. A.*, 1944, 124, 1103–1109.

12. de Winiwarter, H. Recherches sur l'histogenèse du glomérule rénal. *Arch. de biol., Paris*, 1943, 54, 207–224.

13. Dunihue, F. W., and Candon, B. H. Histologic changes in the renal arterioles of hypertensive rabbits. *Arch. Path.*, 1940, 29, 777–784.

14. Dexter, L., Frank, H. A., Haynes, F. W., and Altschule, M. D. Traumatic shock. VI. The effect of hemorrhagic shock on the concentration of renin and hypertensinogen in the plasma in unanesthetized dogs. *J. Clin. Investigation*, 1943, 22, 847–852.

15. Dexter, L., and Haynes, F. W. Relation of renin to human hypertension with particular reference to eclampsia, pre-eclampsia and acute glomerulonephritis. *Proc. Soc. Exper. Biol. & Med.*, 1944, 55, 288–290.

16. Keele, C. A., and Slome, D. Renal blood flow in experimental "crush syndrome." *Brit. J. Exper. Path.*, 1945, 26, 151–159.

17. Eggleton, M. G., Richardson, K. C., Schild, H. O., and Winton, F. R. Renal damage due to crush injury and ischaemia of the limbs of the anaesthetized dog. *Quart. J. Exper. Physiol.*, 1943, 32, 89–106.

18. Duncan, G. W., and Blalock, A. The uniform production of experimental shock by crush injury: possible relationship to clinical crush syndrome. *Ann. Surg.*, 1942, 115, 684–697.

19. Smith, H. W. Lectures on the Kidney. Porter and Welch Lectures, Series 9. University Extension Division, University of Kansas, Lawrence, Kansas, 1943.

19a. Richards, A. N. Cited by Smith.[19]

20. Scarff, R. W., and Keele, C. A. The effects of temporary occlusion of the renal circulation in the rabbit. *Brit. J. Exper. Path.*, 1943, 24, 147–149.

21. Houssay, B. A., and Dexter, L. The sensitivity to hypertensin, adrenalin and renin of unanesthetized normal, adrenalectomized, hypophysectomized and nephrectomized dogs. *Ann. Int. Med.*, 1942, 17, 451–460.

22. Eggleton, G. Crush kidney syndrome in the cat. *Lancet*, 1944, 2, 208–210.

23. Bywaters, E. G. L., and Stead, J. K. The production of renal failure following injection of solutions containing myohaemoglobin. *Quart. J. Exper. Physiol.*, 1944, 33, 53–70.

[*Illustrations follow*]

DESCRIPTION OF PLATES

PLATE 87

FIG. 1. Ischemic glomerular tuft (case A-1, crush wound). Longitudinal section of part of the mesangium supporting the loops, showing increase of the collagen. Cell expansions of the mesangium cells have disappeared. In the loops, some of which are retracted and others relaxed, may be noted coagulated plasma (after fixation), shrunken, hemolyzed red blood cells, one enlarged endothelial cell, and a cluster of thrombocytes. Fixative, 10 per cent formalin. Masson's trichrome stain.

FIG. 2. Juxta-glomerular apparatus seen in longitudinal section and connected with the macula densa (case A-1, crush wound). The microcellular constituent is composed of spindle-shaped cells and is situated in the angle formed by the glomerular arterioles. The macrocellular constituent is composed of afibrillar cells and has a variable cytologic aspect (granular or vacuolar, or in regression). There is fresh subendothelial vacuolar transudate. Shrunken, hemolyzed red blood cells are seen in the infundibulum of the glomerulus. Fixation, 10 per cent formalin. Masson's trichrome stain.

FIG. 3. Juxta-glomerular apparatus seen in transverse section and connected with the macula densa (case A-5, crush wound), showing intense vacuolation of the granular afibrillar cells, vacuolar complexes (Dunihue and Candon [13]), and fresh subendothelial transudate. A capillary may be seen close to the group of granular cells. Fixative, 10 per cent formalin. Masson's trichrome stain.

FIG. 4. Tangential section of a pre-glomerular arteriole in the outer zone of the renal cortex (case 76, traumatic uremia observed in 1914–18). There is a granular transformation of the media. Afibrillar muscle cells show variable cytologic features and contrast with the ordinary smooth muscle cells. Bouin's fixative. Masson's trichrome stain.
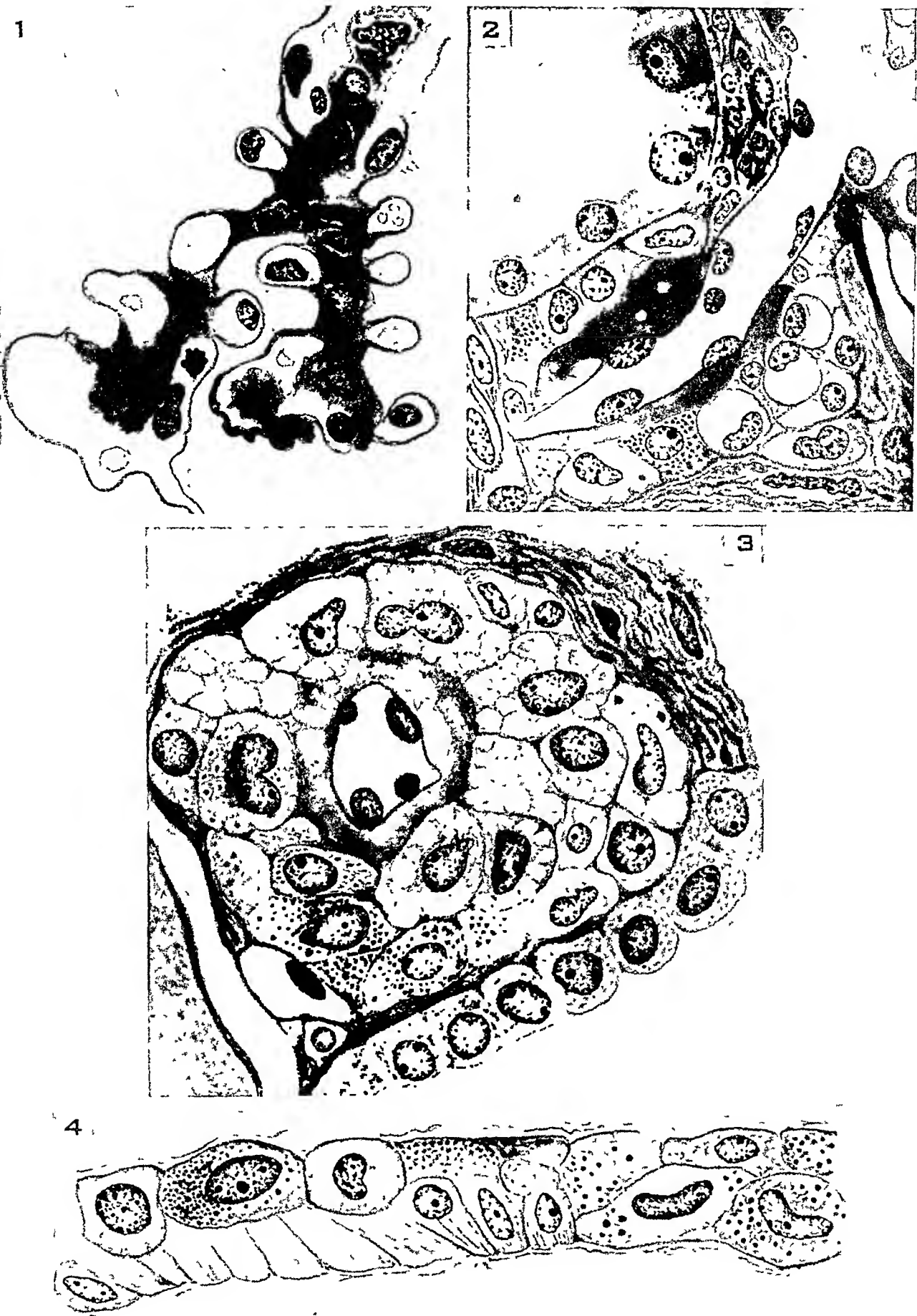
Goormaghtigh

Renal Arteriolar Changes in Crush Syndrome

FIG. 5. Juxta-glomerular apparatus seen in longitudinal section and connected with the macula densa (case 76). There is hypertrophy of the afibrillar cells, some of which contain coarse siderophilic granules. One afibrillar cell protrudes into the lumen of the arteriole. Glomerular ischemia and interstitial edema are present. Flemming's fixative. Heidenhain's iron hematoxylin stain.

FIG. 6. Juxta-glomerular apparatus in tangential section (case A-4, crush wound), showing considerable hypertrophy and granularity of the afibrillar cells, cellular increase in the wall of the infundibulum, and vacuolation of the cells of the macula densa. The contrast may be noted between the latter cells and the epithelium of the proximal convoluted tubule. Fixation, 10 per cent formalin. Masson's trichrome stain. .

FIG. 7. Case A-1. Intertubular capillaries, some showing stainable plasma. Interstitial connective tissue is edematous. Fixation, 10 per cent formalin, Masson's trichrome stain.

STUDIES OF THERMAL INJURY

Parts I, II, and III of Studies of Thermal Injury by A. R. Moritz, F. C. Henriques, Jr., and associates will appear in *The American Journal of Pathology* as follows:

I. Henriques, F. C., Jr., and Moritz, A. R. Studies of Thermal Injury. I. The Conduction of Heat to and through Skin and the Temperatures Attained Therein. A Theoretical and an Experimental Investigation. This issue, pages 531–549.

II. Moritz, A. R., and Henriques, F. C., Jr. Studies of Thermal Injury. II. The Relative Importance of Time and Surface Temperature in the Causation of Cutaneous Burns. September, 1947.

III. Moritz, A. R. Studies of Thermal Injury. III. The Pathology and Pathogenesis of Cutaneous Burns. An Experimental Study. November, 1947.

—*Editor*

I. The Conduction of Heat to and through Skin and the
Temperatures Attained Therein. A Theoretical and an
Experimental Investigation *

F. C. Henriques, Jr., Ph.D., and A. R. Moritz, M.D.

(*From the Department of Legal Medicine, Harvard Medical School, Boston, Mass.*)

I. Introduction

Never before in the history of man has heat energy been so important a cause of disability and death as during World War II. The need for precise knowledge regarding the thermal tolerances of living tissue and the nature of the cellular and somatic changes induced by hyperthermia was felt by those responsible not only for the protection and medical care of our own military personnel but also for the development and use of such thermally effective weapons as the flame thrower, the atomic bomb, and various other incendiary or explosive missiles.

Although thermal injury has always constituted a problem of medical importance, the requests of various branches of the Armed Forces for precise and quantitative information disclosed a dearth of basic facts relating to the casualty-producing effectiveness of heat energy. Remarkably little information was available concerning the mechanism by which hyperthermia leads to irreversible cellular injury, the reciprocal relationships of time and temperature in the production of either cutaneous or systemic injury, the relationship between environmental heat, surface temperature and the slope of the transcutaneous thermal gradient, the pathogenesis of cutaneous burns, or the physiological mechanisms by which external heat may be responsible for acute disability or death.

In an attempt to elucidate some of these problems and to satisfy some of the more pressing needs of the Armed Services for quantitative data, a series of studies was undertaken. It became apparent that the information thereby acquired was of such fundamental importance to an understanding of the problem of thermal injury from a civil as well as from a military standpoint that its publication in the open literature was authorized.

This report, which is the first of a series, is concerned in part with a general consideration of the physical laws that relate to heat transfer and temperature change and in part with a quantitative experimental study of the rate of heat transfer through skin at different surface temperatures and the effects of different rates of energy transfer on the subsurface temperature gradient.

II. THEORETICAL CONSIDERATIONS.

THE NATURE OF HEAT

The concept of temperature arises from the sensations of hotness and coldness. Experience has shown that when a group of substances of different temperature are kept free of outside disturbances, the hotter bodies will get colder and the colder bodies hotter, and, ultimately, these substances will reach a state of complete thermal equilibrium (identical temperature). The hotter bodies are said to have lost heat and the colder bodies are said to have gained heat. This concept of heat becomes quantitative by defining a unit of heat, the calorie, as the amount of heat gained by 1 gm. of liquid water under atmospheric pressure when the temperature increases from 14.5° to 15.5°C.

A gain in heat, which is discernible through a rise in temperature, is associated with an increase in the intra-molecular and inter-molecular motion. Thus, heat can be considered as the energy stored in a substance by virtue of the state of its molecular motion. Certain manifestations of this increase in energy are readily observable; for example, melting, vaporization, decomposition, and alteration in rate of diffusion and chemical reaction.

Beside the definition of a calorie, there are other physical concepts pertaining to heat which are requisite to an understanding of the general problem of thermal injury, namely, heat capacity and heat transfer.

Heat Capacity, C_p

Heat capacity, or specific heat, of a substance is the amount of heat which is required to raise its temperature by 1°C.

The importance of heat capacity in relation to thermal injury is readily seen by considering the respective propensities for production of injury of 1 gm. of water ($C_p = 1.00$) and 1 gm. of silver ($C_p = 0.06$) both at 100°C. and in contact with 1 gm. of thermally insulated skin ($C_p \approx 0.7$) at 35°C. After equilibrium is reached in the former case, the temperature of the skin is increased to 73°C., whereas in the latter case it is increased only to 42°C.

It is apparent that if the skin were to equilibrate rapidly enough

when placed in contact with a hot body, there is insufficient heat in 1 gm. of silver at 100°C. to produce injury to 1 gm. of skin. Actually, of course, the skin, due to its thermal insulating properties, does not equilibrate rapidly enough and the portion of skin nearest the silver does reach a sufficiently high temperature to produce injury before thermal equilibrium is reached. Hence, another physical property of importance in the problem of thermal injury is heat transfer.

Heat Transfer

In certain experiments to be described in ensuing articles,[1,2] heat was transported to the skin by three methods: convection, radiation, and conduction. With convection and radiation,[1] heat reached the skin under such circumstances that the uptake of heat was primarily determined by the heat source. With conduction,[2] the amount of heat absorbed by the skin was primarily determined by the properties of the heat absorber, the skin itself.

Convection. Convection is the mechanism by which hot air transports heat to a cooler surface due to the eddying currents that arise. The air velocities of the eddy currents are about 1.6 km. per hour. An equation has been developed for the transfer of ambient heat by natural convection from a large envelope of hot air surrounding cylindrical objects about 30 cm. in diameter.[3,4] This equation shows that q, the caloric uptake per minute per sq. cm. of surface, can be expressed as follows:

$$(1) \qquad q = 0.0026 \, (T_a - T)^{\frac{5}{4}}$$

where T_a is the air temperature in °C. and T is the surface temperature in °C. Thus, using a skin temperature of 40°C., air at 100°C. and at 400°C. will transport to the skin about 0.4 and 4 calories per sq. cm. per minute, respectively. It is also apparent that as this heat is absorbed by the skin, the surface temperature of the skin will rise and the caloric uptake of the animal will decrease with time.

It is of interest to compare the caloric uptake rate of skin at 40°C. when an atmosphere of steam maintained at 100°C. is substituted for the air. Under these conditions, about 300 calories per sq. cm. per minute would be absorbed by the skin,[3] if the surface temperature could be maintained at 40°C. This 800-fold increase in caloric bombardment as compared to air is due to the latent heat of condensation of steam. This, of course, is why steam is an enormously greater hazard than hot air in the production of heat injury.[5]

Radiation. All substances give off heat in the form of radiant energy

in amounts that are predetermined by their surface temperatures. When this radiation impinges upon another body a certain fraction is absorbed and is changed into heat. Thus, if two substances at different temperatures are placed in an enclosure, there is a continual exchange of energy; the hotter body radiating more energy than it absorbs and the colder body absorbing more heat than it radiates. In the special case of an animal completely enclosed in a large box of source temperature, T_r, in °C., the caloric uptake rate, q, of the animal, due to this interchange of radiant energy between the skin and the wall of the box, is expressed by the following equation: [3,4]

$$(2) \qquad q = sef \left[(T_r + 273)^4 - (T + 273)^4 \right]$$

where s is the radiation constant and is equal to 8.2×10^{-11} calories per sq. cm. per T^4 per minute, e is the effective emissivity of the hot walls of the box, and f is the absorptivity of the skin to radiation emitted at T_r. Under experimental conditions to be described, the product, ef, can be taken as about 0.8. Thus, when the skin temperature is 40°C., the hot walls at 100° or 400°C. will radiate to the skin about 0.7 or 13 calories per sq. cm. per minute, respectively.

Conduction. Conduction is defined as the transfer of heat from the hotter portion of a substance to a colder portion of the same substance, or from a hot body in physical contact with a cold body, when in each case there is no appreciable displacement of any of the molecules comprising these substances. It is this restriction that differentiates conduction from convection.

In certain experiments to be described, heat was conducted from either a hot solid or a hot liquid[2] to the skin. In these experiments, the purpose of both the solid and liquid heat source was to maintain the temperature of the skin surface at a predetermined constant value and hence the conduction of heat through the heat source need not be considered. In hot air experiments,[1] thermal conduction through air is small as compared to convection, and this small contribution is included in equation 1. Thus, conduction of heat through the skin only need be considered.

In all cases of heat flow by conduction, a temperature gradient must exist within the substance. If this temperature gradient varies with time, the rate of heat flow will also vary with time. This type of heat flow in which temperature is a function of both position within the body and time is called heat conduction in the unsteady state. Heat conduction in the steady state refers to all cases in which the temperature at any point within a substance does not depend upon time. Under these conditions the amount of heat flow through the medium is

determined by this temperature gradient and by the ability of the body to conduct heat (thermal conductivity). It is the latter case which will be considered first. The equation for steady state heat conduction inside a rectangular homogeneous body is based upon Fourier's law [3,4,6] and is given by:

$$(3) \qquad\qquad q = \frac{K}{L}(T_1 - T_2)$$

where K, the thermal conductivity, is expressed in calories per minute per sq. cm. perpendicular to the direction of heat flow per unit temperature gradient, °C. per cm. length of path. L is the path length through which the heat flows and T_1 and T_2 are the temperatures in °C. at the beginning and end of the path respectively; q has been previously described.

This equation makes possible the experimental determination of the *in vitro* thermal conductivity of the four respective sections of cutaneous and subcutaneous tissues: epidermis, dermis, fat, and muscle, and also of any combination thereof.

GENERAL THEORY OF HEAT FLOW THROUGH SKIN

By making use of the above brief definitions of the various physical factors involved in the transport of heat to and through the skin, it is possible to consider how the application of heat affects the time-temperature relationship within a given cutaneous site. It is apparent that in order to make heat flow inward from the skin surface it is necessary to raise the temperature of the skin surface to an extent that overcomes the normal existing gradients. This can be accomplished by means of an external source of heat through conduction, convection, or radiation. Once the temperature of the skin surface is sufficiently high, the heat will start to flow inward, resulting in a general rise in temperature within the skin site.

This initial heat flow inward, *and thus the rate of temperature rise within*, will depend primarily upon two physical factors: (a) the heat capacity of the skin or the ability of the skin to absorb the heat, and (b) the thermal conductivity of the skin or the ability of the skin to transport the heat. After a certain interval of time the amount of heat entering the skin site will be balanced by the amount of heat leaving the skin site, and the skin will be "heat saturated." In this state, the new temperature distribution within the skin site will become nearly invariant with time, and the amount of heat flowing through the skin will depend only upon (b) and the skin surface temperature.

It is to be recognized that the above picture involves not only the so-

lution of the steady state of heat conduction, but also the solution of the initial unsteady state of heat flow. In order to solve even the above "idealized" picture, it would be necessary to know the initial temperature gradients within the tissues, the thicknesses, densities, thermal conductivities, and heat capacities of the various layers, and the skin surface temperature as a function of time.

The solution of such a problem involves the following Fourier heat equation:[6]

$$(4) \qquad \frac{K}{PC_pL^2} \left(\frac{d^2T_{xt}}{dx^2} \right) = \frac{dT_{xt}}{dt}$$

where T_{xt} is the temperature at the time, t, at a distance, x, within the skin measured from the skin surface. P is density, and the remaining symbols have been previously defined.

The solution of equation 4, subject to the above-mentioned conditions, is exceedingly complicated. Yet, superimposed upon this are the numerous indeterminate *in vivo* factors which arise when we go from the "idealized picture" to the living animal. It is useful to enumerate the most important of these various indeterminate factors:

(a) Site variations in the respective thickness of epidermis, dermis, fat, and muscle.

(b) Variation of existing temperature gradients within the skin with respect to time and/or position of site.

(c) Unknown average rate of blood flow through the various skin layers, and the unknown variations of the unknown rate of flow with respect to position of site and temperatures within the site.

(d) The appearance of edema fluid in variable quantities which brings forth indeterminate alteration in the density, heat capacity, thickness, and thermal conductivity of the various layers of skin so affected.

It is obvious from the above discussion that any general solution of the time-temperature relationship within a skin site, when heat is applied, is not possible. However, with certain of the experiments to be described in detail,[1,2] it is possible *to derive to a first approximation the time-temperature relationship in the layer of basal epidermal cells.*[*] These experiments were either (i)[2] conducted so as to bring the skin surface immediately to, and maintain it at, a predetermined temperature level until the threshold of irreversible epidermal injury was reached; or (ii)[1] the entire animal was surrounded com-

---

* Actually, these time-temperature relationships can also be estimated at either the skin surface or at any distance within the epidermis. The basal epidermal layer has been specifically chosen since increases in temperature of these cells are of the most import in the production of epidermal injury by heat.

pletely by an envelope of ambient (air) and radiant heat. These experimental conditions at the boundary of the skin surface and source of heat are expressed by the following equation:

(5) $q = H\,(T_s - T)$

where q and T have been previously defined (equations 1 and 2). T_s is the temperature of the heat source in °C. and H, in calories per sq. cm. per minute, is known as the heat transfer coefficient. Conditions under experiments i were tantamount to an infinite heat transfer coefficient ($H = \infty$); and with experiments ii the heat transfer coefficient is finite and the numerical value is readily obtained by combining the radiant and ambient contributions to heat transfer coefficient as computed by equations 1 and 2, respectively. In order to solve equation 4 under the boundary condition expressed by equation 5, it is necessary to assume that the ratio of the total tissue thickness to the epidermal thickness (about 80 μ) is infinite rather than finite. This assumption will lead to slightly longer time intervals for "heat saturation" of the epidermis than are to be experimentally expected. The integration [6] of equation 4 under the above conditions results in equation 6:

$$\frac{T_s - T_t}{T_s - T_o} = \theta\left[\frac{\gamma}{\sqrt{t}}\right]\left\{ e^{\frac{HL}{K}\left(1 + \frac{HLt}{4\gamma^2 K}\right)}\right\}\left\{ 1 - \theta\left[\frac{\gamma}{\sqrt{t}}\left(1 + \frac{HL}{2\gamma^2 K}\right)\right]\right\}$$

where

(6a) $\theta\,[Y] = \frac{2}{\sqrt{\pi}}\int_0^Y e^{-x^2}dx$

and γ is computed by means of equation 6b.

(6b) $\gamma = \dfrac{L}{2\sqrt{\dfrac{K}{PC_p}}}$

T_t is the temperature of the basal epidermal cells at the time, t, in seconds. T_s is the temperature of the heat source. T_o is the temperature of the skin surface previous to the exposure to heat. L is the distance of the basal cells from the skin surface. P is the density of the basal epidermal layer. The other symbols have been previously defined and are experimentally determinable. The integral that defines $\theta\,[Y]$ (equation 6a) is respectively equal to $\sqrt{\pi}/2$ and zero when Y is infinite ($t = 0$) and Y is zero ($t = \infty$). For other values of Y, the numerical value of the integral is tabulated.[7]

The time-temperature relationships at the basal-epidermal layer

during an exposure of the animal to a source of constant ambient and radiant heat will be evaluated by means of these equations in section IV.

In experiments in which the skin surface was brought immediately to, and maintained at, a predetermined constant temperature, H, the heat transfer coefficient, is nearly infinite, and equation 6 reduces to

$$(6c) \qquad \frac{T_s - T_t}{T_s - T_o} = \theta\left[\frac{\gamma}{\sqrt{t}}\right]$$

where, as before, γ is given by equation 6a. It is to be noted that in this case T_s can be taken as the skin surface temperature during the entire heat exposure, since the temperature of the heat source is identical with the surface temperature once heat exposure begins.

Equation 6c results in a basal layer temperature which becomes, after a certain time interval, essentially identical with the skin surface temperature. Actually, there will always exist a small but finite temperature gradient between the surface and the basal cell layer. This steady state gradient can be experimentally determined by means of equation 3, and the true temperature of the basal layer can be quite accurately computed for any time, *t*, by using equation 6c until the steady state temperature obtained through equation 3 is reached. Computations using equation 6 to ascertain basal epidermal temperatures will be given in section IV.

III. An Experimental Investigation of the Quantities Involved in Both the Steady and Unsteady State of Heat Conduction Through the Skin

Determination of the Heat Capacity of the Four Pertinent Tissues

The apparatus used for the determination of the heat capacity of these cutaneous and subcutaneous tissues need not be described in detail, since the well known method of mixtures was used.[8] Briefly, this procedure consisted of heating a known weight (about 10 gm.) of each tissue in a thin brass container and rapidly dropping it into a water calorimeter. The heat capacity of the tissue was readily computed from temperature rise of the water as measured by a Beckmann thermometer.

In order to obtain pure epidermis for these determinations, the following method was used. After the hair was shaved as closely as possible, the pig was immersed in the water at 55°C. for about 1

minute, then withdrawn and the skin carefully dried. It was then possible to remove strips of pure epidermis by scraping with a knife. The remaining tissues were readily obtained in a relatively pure state by dissection.

The values of the heat capacities of the respective tissues of two pigs are given in Table 1.

TABLE I

Heat Capacity of Cutaneous and Subcutaneous Tissues of the Pig in Calories per Gram per °C.

	Epidermis	Dermis	Fat	Muscle
Heat capacity, C_p	0.887 0.845	0.785 0.753	0.538 0.573	0.890 0.926
Average value, $\overline{C_p}$	0.86	0.77	0.55	0.91

In view of the known similar heat capacities of dry tissue, the variations of the different tissues as shown in Table 1 are probably due to the water content. In this respect, the high value for epidermis (0.86) is understandable since it was found experimentally that the water content of epidermis, in spite of the presence of the cornified layer, averaged about 76 per cent.

Determination of the Thermal Conductivities of Cutaneous and Subcutaneous Tissues of the Pig

The experimental determinations of the thermal conductivities of cutaneous and subcutaneous porcine tissue were based on equation 3. The respective tissues were placed on a copper cylinder, 5 cm. in diameter and 10 cm. high. The temperature of this tissue-cylinder interface was measured by means of an iron-constantan thermocouple soldered into the face of the copper cylinder and a type K2 potentiometer.* The automatic recording caloric applicator [9] was then placed over, and in contact with, the exposed face of the tissue. This apparatus provided a means of automatically recording the vertical rate of caloric flow from the applicator through the tissue to the copper cylinder as functions of the temperatures of both tissue surfaces. Thus, when the tissue became "heat saturated," knowledge of the caloric input into the tissue, of the temperatures of the tissue-applicator (about 48°C.) and of tissue-cylinder (about 30°C.) interfaces, and of the thickness of the tissue made possible computation of thermal conductivity. The average tissue thickness was determined

* Leeds & Northrup, Philadelphia, Pa.

by measuring the distance between the face of the applicator and the face of the cylinder. The thermal conductivities of all tissues except epidermis were obtained by this procedure, since in view of the thinness of epidermis the above method was not feasible.

The method of difference was used with epidermis. A section of well shaved skin tissue consisting of dermis and epidermis was rigidly clamped to the copper cylinder, water at 55°C. was poured over the skin, and the excess water was removed by blotting. The clamps prevented lateral contraction of the heated tissue and the hot water facilitated subsequent removal of the epidermis. The conductivity determination was then made, the epidermis scraped off, and the determination repeated. As a further check, in certain experiments a

TABLE II

In Vitro Thermal Conductivities, K, of Pig Tissues

$$K = \frac{calories \times cm.}{cm.^2 \times minutes \times °C.}$$

	Epidermis	Dermis	Subcutaneous fat	Subcutaneous muscle
K	0.036	0.054	0.021	0.064
K	0.023	0.053	0.024	0.062
K	0.032	0.051	0.023	0.073
K	0.03	0.053	0.023	0.066

strip of intact epidermis was placed over the denuded dermis and the measurement repeated. The thickness of numerous pig epidermal strips was determined with a micrometer. The thickness was about $80 \pm 10 \, \mu$.

At least triplicate determinations were made on each of the four tissues of three different pigs (about 10 kg.). The average values of the thermal conductivities obtained are given in Table II.

In view of the thinness and uncertainty as to thickness of the epidermis, the wide variation in the epidermal thermal conductivity was to be expected. The data pertaining to the other tissues were considerably more reproducible.

It is of interest to compare some of these data with those of Breuer,[10] who determined the respective thermal conductivities of both muscle and fat of cow, horse, pig, and dog. This investigator found that the conductivities of pig muscle and fat, expressed in the above units, were 0.060 and 0.021, respectively; furthermore, essentially the same values were found for the muscle and fat of the other three animals. In view of the excellent agreement between Breuer's value and ours for pig muscle and fat, it is difficult to understand the value, 0.03, that Hardy and Soderstrom [11] reported for both cow muscle and fat. Un-

fortunately, no description of their experimental method was given. In order to investigate this discrepancy, the thermal conductivity of beef muscle was redetermined and an average value of 0.057, which agrees with the results of Breuer, was obtained.

In view of the numerous indeterminate factors (section II) which enter into the *in vivo* conduction of heat through pig skin, the *in vitro* thermal conductivities of these four tissues are not of themselves very useful. However, they do serve as a baseline in the interpretation of certain experiments to be described.

In Vivo Observations of Caloric Uptake of Pig Skin and the Rise in Temperature at the Dermis-Fat Interface as a Function of Both Time and Skin Surface Temperature

It was of interest to ascertain the caloric uptake of the skin when the epidermal surface was maintained at various temperature levels between 45° and 100°C. Numerous such experiments have been performed and, as was to be expected (see section II), the data varied widely and were extremely difficult to interpret in detail. Therefore, only a small fraction of these data will be reported and the variations to be expected will be indicated.

During these experiments the temperature at the dermis-fat interface also was ascertained. A pig under nembutal anesthesia was clipped and shaved. The no. 27 gauge needle thermocouple[9] was introduced laterally into the dermis-fat interface in the following manner: Through experimentation it was found possible to insert laterally a no. 22 gauge trocar along the natural cleavage plane of the dermis-fat interface, until a point directly underneath the surface area to be exposed to heat was reached; then the no. 27 gauge thermocouple needle was inserted into the no. 22 gauge trocar until skin resistance could be detected. The no. 27 gauge needle was then withdrawn about 1 cm. The temperature (millivolts) of this needle couple was measured either intermittently with a type K2 potentiometer * or continually with a photo-electric recording potentiometer.†

After the determination of the skin temperature at a chosen site by means of the fine wire thermocouple,[9] the automatic recording caloric applicator[9] was applied, and a continuous record of the caloric uptake rate of the skin at a predetermined epidermal surface temperature was obtained.

After the heat exposure was terminated, the skin was cut to expose the hypodermic needle thermocouple and the distance from the dermis-

---

* Leeds & Northrup, Philadelphia, Pa.
† General Electric Co., Schenectady, N. Y.

fat interface to the skin surface was ascertained with a depth gauge. This depth before the application of heat was ascertained by control experiments on neighboring sites.

Caloric Uptake Rate of Pig Skin

Typical caloric uptake data as a function of time and epidermal surface temperature are presented in Table III. These data were a

TABLE III

A Guide (± 30 Per Cent) to the Caloric Uptake of Pig Skin as a Function of Time and Surface Temperature as Determined by the Automatic Recording Caloric Applicator[9]

| Time interval | Skin surface temperature during heat exposure | | | | |
	45° C.	50° C.	55° C.	60° C.	65° C.
(minutes)	Caloric uptake rate of lateral thoracic skin in calories per minute per sq. cm.				
0 −0.2	6.0	9.5	12.0	15.0	17.0
0.2−0.4	2.2	3.7	4.9	6.9	8.4
0.4−0.6	1.8	2.8	3.8	5.6	6.7
0.6−0.8	1.7	2.5	3.1	5.1	5.9
0.8−1.0	1.6	2.3	2.7	4.7	5.4
1 −1.5	1.5	2.0	2.6	4.3	4.8
1.5−2	1.4	1.8	2.4	4.1	4.5
2 −3	1.2	1.6	2.3	3.7	4.2
3 −5	1.1	1.5	2.1	3.2	3.8
5 −7	1.0	1.4	2.0	2.8	3.5
7 −10	0.9	1.3	1.9	2.5	3.2
	Total caloric uptake in calories per sq. cm.				
0 −1	2.7	4.2	5.3	7.5	8.7
0 −5	7.5	10.7	14.3	21.8	25.2
0 −10	12.2	17.4	24.0	34.9	42.7

composite of at least three determinations on the lateral thoracic area of different pigs; five pigs in all were used. As was expected, in view of the numerous factors that determine the caloric uptake in a living animal (section II), the experimental variations inherent in duplicating exposures were considerable. Thus, these data served only as a rough guide (± 30 per cent) to the caloric uptake rate of pig skin.

It was noted that the average caloric uptake rate of pig skin during the first 0.2 minute was about six-fold greater than the average caloric uptake rate during the steady state period (7 to 10 minutes). This six-fold difference was due to the initial necessity of heat saturating the tissue and was primarily a heat capacity effect. After the first few minutes the skin tissue was essentially heat saturated and the *in vivo* thermal conductivities of the various layers of pig skin primarily determined the caloric uptake rate.

When the data given in Table III are plotted against time, the curves obtained will conform in type to that shown schematically in Text-Figure 1. A mathematical analysis of the general form of these curves based on equation 5 showed that during the first 2 to 3 minutes of the heat application, the skin can be considered as an infinite body with a ratio of thermal conductivity to heat capacity that was approximately

the same as that computed from the *in vitro* determinations tabulated in Tables I and II. This agreement was probably due to the fact that the ratio of the thermal conductivity to heat capacity (equation 6b) was not nearly as sensitive to these indeterminate *in vivo* factors as the individual quantities themselves.

The Interface Temperature at the Junction of Dermis and Fat

The dependence upon time of the dermis-fat interface temperatures during exposure of the skin surface to a predetermined temperature between 45° and 90°C. is given in Table IV. These values were a composite of at least two experimental determinations on two different pigs (four determinations in all). As with measurements of the caloric
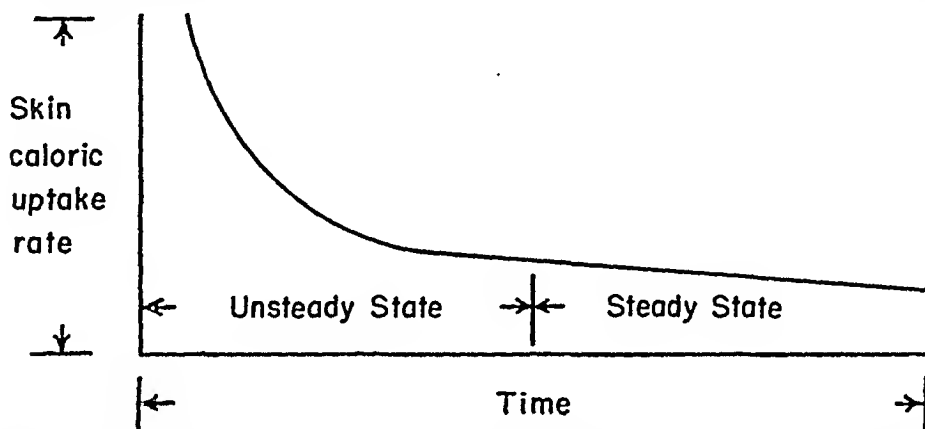
Text-Figure 1. The time dependence of the rate of heat flow through skin when the surface is immediately brought to, and maintained at, a constant temperature.

uptake, the variations in duplicating experiments were considerable, and these data serve only as a rough (± 20 per cent) guide to the time-temperature relationship at the dermis-fat interface. These data together with other experimental observations indicate the following:

(a) The junction of the fibrous dermis and the subdermal fat in the lateral thoracic area of a 10 kg. pig lay about 2 mm. below the skin surface. Ten minute exposures to surface temperatures of 50° to 70°C. increased, significantly, the thickness of the dermis. This increase in thickness was due to the accumulation of edema fluid in the dermis and the effect was maximal when the skin surface was maintained at about 60°C. Skin surface temperatures equal to, or greater than, 80°C. denatured the corium so rapidly that these mechanisms were destroyed.

(b) Although the continual caloric uptake by the skin tended to increase the dermal temperature, the appearance of relatively cool edema fluid tended to decrease it. At skin surface temperatures of 50° and 70°C. these two effects nearly counterbalanced, and after the first minute of heat exposure, the dermis-fat interface temperature remained essentially constant. With skin surface temperatures between 55° and 65°C. the rapid appearance of a large amount of edema

fluid more than compensated for caloric uptake, and the temperature at the interface between dermis and fat was temporarily lowered. This effect was maximal when the skin surface was maintained at about 60°C.

(c) When the skin surface temperature was maintained at 45°C., and probably at all other temperatures that fail to cause edema, the dermis became "heat saturated" after about 5 minutes of exposure.

TABLE IV

The Dependence on Time of the Dermis-Fat Interface Temperature ($\pm$ 20 Per Cent) During Exposure of the Surface of Pig Skin to Predetermined Temperatures

Initial	Skin surface temperature in °C.							
Initial	35.0	34.8	34.8	35.2	34.9	34.3	34.2	34.5
During exposure	45	50	55	60	65	70	80	90
Time	Dermis-fat interface temperature in °C.							
(minutes)								
	34.7	34.5	34.6	35.0	34.7	34.2	34.4	34.8
0.2	36	38	39	43	46	52	53	56
0.5	38	43	45	46	52	62	65	66
1.0	39.5	45	47	48	53	65.6	71	74
1.5	40	47	48	47	53	66.5	72	77
2.0	40.5	47	49	46	54	67	74	79
3.0	41	47	48.5	45	56	67.5	75	79
5.0	42	47	47.5	44.5	58	67.5	77	
7.0	42	46.5	47.5	47	58			
10.0	42	47	48	49	59			
Initial	Average thickness of corium in mm.							
Initial	2	2	2	2	2	2	2	2
At termination of exposure to heat	2	2.5	3.2	4.2	3	2.5	2	2
	In vitro thermal conductivity of dermis at termination of exposure							
	0.06	0.1	0.09	0.10	0.16			

When edema fluid was produced, the time for dermal heat saturation was essentially indeterminate, but it apparently was not much greater than 10 minutes.

(d) Histological examinations showed that complete primary injury to the dermis immediately following heat exposure was obtained in all of these experiments when the skin surface temperature was maintained at 65°C. or above. The limited (five) time-temperature-injury data at the dermis-fat interface tended to indicate a quantitative relationship not at variance with that found for epidermal injury.[2,12]

(e) By making the reasonable assumption that the dermis was essentially "heat saturated" at the end of a 10 minute exposure, the *in vitro* thermal conductivities of dermis can be computed by substituting the

approximate caloric uptake (Table III), dermis-fat interface and skin surface temperatures, and the final dermal thickness in equation 3; neglect of the epidermal temperature drop introduced no appreciable error. Table IV also shows the results of these calculations. A comparison of these values with the experimentally determined *in vitro* value of 0.053 (Table II) for pig dermis indicates that the presence of edema fluid increased the thermal conductivity of dermis two- to three-fold. This increase in conductivity, however, was slightly more than compensated by the swelling of the dermis, and thus an edematous dermis was a somewhat better heat barrier to the underlying tissues than normal dermis. A comparison of the *in vivo* thermal conductivity of dermis obtained at 45°C. with the *in vitro* value tended to indicate that intact circulation probably increased the effective thermal conductivity of dermis by about 15 per cent.

IV. Estimation of the Temperature Changes at the Epidermal-Dermal Interface During the Exposure of the Skin Surface to Heat

In view of the thinness ($\sim$80 μ) of the pig's epidermis, experimental measurement of the time-temperature relationships at the epidermal-corium junction was not feasible. There are certain facts, however, that allowed the estimation of this time-temperature relationship with a considerable degree of certainty. In view of the extreme thinness of epidermis, the temperature of the basal layer was largely determined by skin surface temperature. This is most readily seen by solving heat conduction equation 3 for the steady state temperature of the basal epidermal layer. Of the four necessary experimental quantities; namely, skin surface temperature, epidermal thickness (about 80 μ), epidermal thermal conductivity (Table II), and caloric uptake of the skin at the requisite skin surface temperature (Table III), only the last two were subject to considerable variation ($\pm$ 30 per cent). Fortunately, even variations of this magnitude result in uncertainties of less than 0.2°C. in the steady state temperature of the basal epidermal layer.

Basal Epidermal Temperatures When the Skin Surface Is Immediately Brought to, and Maintained at, a Temperature between 45° and 100°C.

Before the steady state temperature is attained, the time-temperature relationship at the epidermal-dermal junction is given under these conditions to a good approximation by equation 6c, where γ has the numerical value

$$(7) \qquad\qquad\qquad \gamma = 0.15$$

if the time, t, is expressed in *seconds*.

The numerical constant, 0.15, is not subject to the experimental uncertainties of the quantities requisite to computation by equation 6b, since this value was empirically determined [12] with considerable accuracy from data on experimental temperature-time-epidermal injury.[2] An identical value for γ can be directly computed also by substituting into equation 6b the heat capacity, thermal conductivity, and thickness

TABLE V

The Computed Time-Temperature Relationships for the Epidermal-Dermal Interface When the Skin Surface Is Immediately Brought to, and Maintained at, a Specific Temperature

	Surface temperature during heat exposure				
	45° C.	55° C.	65° C.	80° C.	100° C.
Time	Temperature at basal epidermal layer*				
(seconds)					
0	35.0	35.0	35.0	35.0	35.0
0.01				36.3	37.0
0.02			38.9	40.9	43.4
0.05		41.8	45.2	50.3	57.1
0.1	40.1	45.2	50.3	57.9	68.2
0.2	41.3	47.6	53.9	63.3	75.9
0.5	42.7	50.4	58.1	69.6	85.1
1	43.3	51.6	60.0	72.4	89.1
2	43.8	52.6	61.4	74.6	92.3
5	44.2	53.5	62.7	76.6	95.1
10	44.5	53.9	63.4	77.6	96.6
30	44.7	54.4	64.1	78.6	98.0
60	44.8	54.6	64.4	79.0	98.6
120	44.9	54.9	64.5	79.4	99.2
300	44.9	54.9	64.7	79.5	99.3
600	44.9	54.9	64.8	79.7	99.6
Steady state†	44.8	54.5	64.2		

* Computed by means of equations 6b, 6c, and 7.
† Computed by means of equation 3 and experimental data of Table II.

of epidermis, and assuming an epidermal density of 0.8 gm. per cc., a most reasonable value. In view of the two completely independent methods, one of which was *in vivo* and the other *in vitro*, considerable confidence can be placed in the adaptation of the "infinite body picture" (section II) to the solution of the time-temperature relationship at the epidermal-dermal junction during the unsteady state period of heat flow.

The computation of the temperature of the basal cell layer of the epidermis as a function of both time and skin surface temperature is given in Table V. These data show that there was a rapid rise in the temperature of the basal epidermal layer when the skin surface was immediately brought to, and maintained at, a specified constant temperature. A comparison of the data of the unsteady state computed from equation 6c with those of the steady state obtained by means of

equation 3 shows that the epidermis under the above conditions became essentially "heat saturated" after a heat exposure of 0.5 to 1.0 minute's duration.

It must be re-emphasized that these data apply only to situations in which the heat transfer coefficient, H, from the temperature source to the skin surface can be considered as infinite. In all cases where H is finite, an analysis similar to that given below is required.

Basal Epidermal Temperatures When the Entire Animal Is Surrounded by an Envelope of Ambient (Air) and Radiant Heat between 80° and 175°C.

In the previous discussion, the time-temperature relationships at the epidermal-dermal junction depended only upon the rate of heat transfer through the skin and the constant temperature of the heat source. To this must now be added the slow rate at which heat is transported from its source to the skin surface via air conduction, air convection, and infra-red radiation. The mathematical solution of this problem is given by equations 6 and 7 where the only quantity that requires further consideration is H, the heat transfer coefficient from the heat source to the skin surface. This quantity is readily computed through the substitution of equation 1, heat transfer by convection, and equation 2, heat transfer by radiation, into equation 5. The numerical values of the heat transfer coefficient which were obtained at particular source or air temperatures are shown in Table VI. A comparison of these

values of H, 0.015 to 0.026 $\dfrac{\text{calories}}{\text{sq. cm. per minute per °C.}}$, with epidermal

thermal conductance, K/L (Table II), numerically equal to 4 in these units, indicates the slow rate at which ambient and radiant heat was transferred to the skin surface as compared to the rate at which this heat flowed through the epidermis.

Table VI gives also the estimated temperature of the basal epidermal layer as function of source or air temperature as calculated by means of equation 6. These data show the extreme slowness of temperature rise at this epidermal-dermal junction. In fact, under these conditions, the epidermal temperature even after a heat exposure of 15 minutes was far lower than the temperature of the heat source, and actually an animal would succumb to hyperthermia[1] long before the temperature of the skin approached that of the air.

Although the data for the time-temperature relationships at the skin surface are not given, they can be readily computed by putting L (the epidermal thickness) equal to zero in equation 6. If this be done, it will be found that except for the first 20 seconds of heat exposure, the

skin surface temperature was not significantly different than the values recorded in Table VI for the basal-epidermal temperature. This is due to the fact that under the conditions specified, heat transfer to the skin was the controlling factor. Thus, these data can be taken also as the temperature of the skin surface as a function of time.

A comparison of Tables V and VI indicates the importance of the mode of imparting heat to the skin surface in the epidermal time-tem-

TABLE VI

The Computed Time-Temperature Relationships at the Epidermal-Dermal Interface When an Entire Animal (~ 30 cm. Diameter) Is Surrounded by an Envelope of Ambient Air and Radiant Heat That Results from a Constant Temperature Source

Time	Circumambient temperature				
	80° C.	100° C.	125° C.	150° C.	175° C.
	Heat transfer coefficient,* H, in calories per sq. cm. per minute per °C.				
	0.015	0.019	0.021	0.024	0.026
	Temperature at basal epidermal layer†				
(seconds)					
0	35° C.	35° C.	35° C.	35° C.	35° C.
10	37	39	40.5	44	46
20				46.5	49
30	38.5	41.5	44	49	52
40				51	54.5
50	39.5	43.5	46.5	53	57
70	40	44	48	56	60
100	41	45.5	50	59	64
130	42	47	52	61	
160	42.5	48.5	54.5	63	
200	43	50	56	65	
300	45	52.5	59		
400	46	55	63		
500	47				
600	48				
800	50				
1000	50.5				
1200	51				

* In order to make these data directly comparable to the published experimental investigations,[2] the radiant contribution to H was computed by using a radiant temperature 20% in excess of the air temperature.

† Computed by means of equations 1, 2, 5, 6, 6a, 6b, and 7. Due to both the thinness of the epidermis and the slow rate of heat transport to and through the skin, there is no appreciable difference between these temperatures and those of the skin surface after the first 20 seconds of heat exposure.

perature relationships. In fact, for a given source temperature, a mechanism that enables the surface temperature to be immediately brought to, and maintained at, the source temperature has, on a time basis, at least a thousand times greater propensity to injure the epidermis than a heat source which raises the skin temperature by means of radiation, and conduction and convection of relatively immobile air.[1,2,12]

V. SUMMARY

The various physical factors which determine the transfer of heat energy to and through the skin and the temperatures attained thereby have been defined and discussed. A general theory of heat flow, which

enabled the estimation of the time-temperature relationships within the epidermis during exposure to heat, was developed.

The thermal conductivities and heat capacities of epidermis, dermis, and subcutaneous fat and muscle were measured *in vitro.*

Experimental observations pertaining to the rate at which thermal energy is taken up by the skin, during surface exposures of varying intensity, and the sub-surface thermal gradients established therein, have been presented.

The time-temperature relationship at the dermal-epidermal junction was computed under two greatly different experimental conditions: (i) when the skin surface temperature was immediately brought to, and maintained at, the temperature of the heat source, and (ii) when the entire skin surface was exposed to specified circumambient and circumradiant temperatures. These data indicate the extreme importance of the mode of applying heat to the skin surface in the time-temperature relationships within the epidermis.

REFERENCES

1. Moritz, A. R., Henriques, F. C., Jr., Dutra, F. R., and Weisiger, J. R. Studies of thermal injury. IV. Exploration of casualty-producing attributes of conflagrations. The local and systemic effects of generalized cutaneous exposure to excessive circumambient (air) and circumradiant heat of varying duration and intensity. *Arch. Path.*, 1947, 43, 466–488.
2. Moritz, A. R., and Henriques, F. C., Jr. Studies of thermal injury. II. The relative importance of time and surface temperature in the causation of cutaneous burns. *Am. J. Path.* (In press.) Moritz, A. R. Studies of thermal injury. III. The pathology and pathogenesis of cutaneous burns. An experimental study. *Am. J. Path.* (In press.)
3. McAdams, W. H. Heat Transmission. McGraw Hill Co., New York, 1942.
4. King, W. J. The basic laws and data of heat transmission. III. Free convection. *Mech. Eng.*, 1932, 54, 347–353.
5. Moritz, A. R., Henriques, F. C., Jr., and McLean, R. The effects of inhaled heat on the air passages and lungs. An experimental investigation. *Am. J. Path.*, 1945, 21, 311–331.
6. Carslaw, H. S. Introduction to the Mathematical Theory of the Conduction of Heat in Solids. Macmillan Co., New York, 1921.
7. Peirce, B. O. A Short Table of Integrals. Ginn & Co., Boston, 1929.
8. White, W. P. Specific heats at high temperatures. *Physical Rev.*, 1908, 26, s. 1, 536–537.
9. Henriques, F. C., Jr. Studies of thermal injury. VIII. Automatic recording caloric applicator and skin tissue and skin surface thermocouples. *Rev. Sci. Instruments.* (In press.)
10. Breuer, H. Ueber die Wärmeleitung des Muskels und Fettes. *Pflüger's Arch. f. d. ges. Physiol.*, 1924, 204, 442–447.
11. Hardy, J. D., and Soderstrom, G. F. Heat loss from the nude body and peripheral blood flow at temperatures of 22° to 35° C. *J. Nutrition*, 1938, 16, 493–510.
12. Henriques, F. C., Jr. Studies of thermal injury. V. The predictability and significance of thermally induced rate processes leading to irreversible epidermal injury. *Arch. Path.*, 1947, 43, 489–502.

A HISTOLOGICAL STUDY OF SKELETAL MUSCLE IN ACUTE ISCHEMIA *

John W. Harman, M.B.†

(*From the Department of Pathology, Wisconsin University School of Medicine, Madison, Wis.*)

The death of cells is a complicated process which may be assumed to proceed in an orderly manner depending upon the initiating cause.[1] In the progress of studies upon voluntary or skeletal muscle it became necessary to make correlation between cell death and its early morphological manifestation. Muscle is of exceptional value, owing to its contractility, as a mechanism in which the morphological alterations constituting necrosis may be studied conveniently. The present investigation undertakes, therefore, to define the early histopathological alterations accompanying the necrosis of ischemic voluntary muscle and to correlate these changes with the times of loss of viability and contractility in this tissue.

Earlier workers,[2-8] because of interest in the pathogenesis of Volkmann's contracture, have devoted most of their attention to the chronic lesions arising from ischemia of voluntary muscle, and have given little attention to the initial phases or changes that occur during the first 24 hours. It has been strongly indicated [7] that the changes peculiar to Volkmann's contracture commence during the first hours of ischemia and are irreversible soon thereafter; however, no record of a detailed study of that initial period has been found. Those who have studied Zenker's degeneration [9-13] have similarly paid little attention to the very early changes and applied themselves to the later manifestations of the lesion or confined their early studies to artificial systems *in vitro*. Indeed, many of these workers have indicated that vascular occlusion causes no change in voluntary muscle even after 18 hours. In studies of skeletal muscle regeneration,[12,14] the phenomena analyzed closely are mainly those that occur after a lapse of 1 or more days following injury or repair. Consequently, despite their importance, the initial lesions and their pathogenesis remain to be described.

METHODS

A state of complete, acute ischemia was induced in the hind limbs of rabbits weighing 2000 to 3500 gm. and in albino rats of the Sprague-Dawley strain with an average weight of 250 gm. In the rabbit the

ischemia was produced in two ways: by ligation of vessels and by application of tourniquets. The animals were operated upon under amytal anesthesia, occasionally supplemented with ether; the common iliac, common femoral, the inferior epigastric arteries and conspicuous branches of the common femoral artery were doubly ligated, under sterile precautions. Ligation of these particular vessels theoretically should preclude intervention of the collateral circulation, such as was described for the hind limb of the normal rabbit,[15] and the efficacy of these ligations was empirically indicated by one observer.[7] By this method a more complete ischemia of certain muscles was obtained than by selective ligation of the arteries to the particular muscles, because even with careful occlusion of the main vessels, a muscle may retain some effective vascularization from its connection with periosteum.[16] In 12 rabbits, including a few weighing 1500 gm., a tourniquet was applied tightly around one thigh so as to occlude arterial blood flow for the desired interval; these animals were also under amytal narcosis. The duration of the experiment was measured from the initial ligation of the common iliac artery, or the application of the tourniquet, until the muscle was removed for fixation. On the other hand, in rats ischemia was induced only by application of the tourniquet to the thigh under nembutal anesthesia. The duration was limited to 12 hours or less because the animal may either mutilate the occluded limb or bite off the tourniquet.

After the required time interval the animals, with further anesthesia when necessary, were prepared for operation. The muscles of both hind limbs were exposed and tested *in situ* for contractility with a faradic current, applied directly to the muscle through metal electrodes, which derived current from a DuBois-Reymond coil, arranged for faradization by a Neef's hammer. The strength of the current was gauged so as to produce a vigorous contraction of the normal limb muscles and was then used to test the contralateral counterpart in the ischemic limb. Contractions were graded as absent, weak, or tetanic, relative to those in the normal limb.

For histological studies the tissues were placed in 10 per cent neutral formalin, absolute ethyl alcohol, and freshly prepared Zenker's solution, at 37° C. Tissues were embedded in paraffin, cut longitudinally, approximately 8 μ in thickness, and stained with hematoxylin and eosin, and Mallory's phosphotungstic acid hematoxylin. The numerical incidence of abnormal muscle fibers was calculated approximately, by a count of 100 fibers in each slide, and represented as a percentage. In all instances comparisons were made between ischemic muscle and the contralateral normal muscle of the same animal. In addition,

teased unfixed fragments of ischemic and normal muscle from rabbits and rats were examined. Several samples of human muscle, taken at necropsy within an hour of death, were also studied.

EXPERIMENTAL OBSERVATIONS

Numerous normal muscles, fixed immediately in either formalin or Zenker's fluid, afforded a picture with which the alterations in ischemic muscle might be contrasted. Certain peculiarities of normal, rapidly fixed skeletal muscle were conspicuous. Of these the most salient was the prominence of longitudinal striations, which contributed largely to the pattern of the fascicles; the fibrils composing the striations were wavy or loosely undulant, usually giving the particular group of fibers a watered-silk appearance (Figs. 1 and 2). In association with this the individual fibers were compactly arranged and so closely approximated that, except for an occasional linear arrangement of nuclei, they frequently could not be distinguished and resembled a syncytium. Because of this I have designated these compact structural forms as "syncytoid." In contrast to the prominence of the longitudinal striations and fibrils, cross striations were inconspicuous. In fact, the definition of the longitudinal and cross striations was reciprocal; the distinctness of the one was proportional to the vagueness of the other.

It may be said, therefore, that the normal mammalian skeletal muscle (rabbit, rat, man) is syncytoid, with conspicuous longitudinal striations and vague cross striations. This differs from the pattern usually regarded as normal, in which the structure of the cross striation is accentuated.

When ischemia was of sufficient duration this pattern was altered in a significant manner. The syncytoid structure was lost early, owing to a departure of the fibers from their collective compact arrangement. They showed widespread individualization and separation by clear structureless spaces (Figs. 3 and 5). The individualized fibers appeared compact and cylindrical. The longitudinal striations in such fibers were vague or absent, and when present were no longer undulant. In contrast the cross striations (Fig. 4) were very manifest and became a dominant feature. With shorter periods of ischemia the finer structure of these cross striations did not differ from that in normal muscle. Following longer periods of ischemia a new type of cross striation appeared with increasing frequency. This differed from the normal, owing to the inordinately great bulk of the anisotropic segment, which was excessively wide and broad in contrast with the narrow isotropic segments (Fig. 6). Eventually the enlarged anisotropic segments assumed the appearance of a series of adherent disks, a feature enhanced

by the splitting away or cracking off of these segments (Fig. 7). The longer the period of ischemia the more widespread and well developed were these changes, particularly those affecting the transverse striations (Fig. 8).

The degree to which changes occurred differed considerably in the various muscles of the ischemic limb (Table I). In the series of rabbits studied the proximal thigh muscles were usually least affected, whereas the flexors and extensors of the leg were always involved to a degree dependent upon the duration of the ischemia. Of the various muscles examined the tibialis anticus reflected most constantly the duration of the ischemia, whereas in the other muscles no regularly reproducible pattern of behavior was demonstrable. In view of the constancy in

TABLE I

The Percentage of Ischemic Muscles Showing Abnormal Fibers in the Rabbit Series

Muscle	No. of muscles examined	No. with abnormal fibers	No. expected with abnormal fibers	Percentage*
Gracilis	11	4	11	36
Adductor magnus	11	2	11	18
Quadriceps	10	3	10	30
Gastrocnemius	20	11	14	78
Tibialis anticus	25	19	19	100

* The percentage is based on the number of muscles involved compared with the number expected to be involved from the duration of ischemia.

gradation of response of the leg muscles, especially the tibialis anticus muscle, this muscle was selected to present the development of the morphological changes due to ischemia, although similar alterations occurred in other muscles, but less frequently.

An alteration in structure was first observed in the muscles of animals subjected to ischemia for at least 4 hours. This was characterized by individualization of fibers, regression of longitudinal striation, and enhancement of cross striation. After 6 hours of ischemia there was, in addition, a change in the type of cross striation to that of abnormal anisotropic disks as described above. The number of fibers composed of such disks increased with the duration of ischemia (Table II), until after 18 to 24 hours nearly all were affected, with considerable fragmentation and splitting off of individual anisotropic disks. The onset of depression of contractility regularly preceded the observed morphological changes to a significant extent. The contractions were vermicular and weak after 2 hours, and entirely absent after 4 hours of ischemia.

On the other hand, in the rat (Table III) these changes were first

seen at the second hour and were well developed, with disk formation by the fourth hour. Thereafter the progression toward more extensive numerical involvement was identical with that in the rabbit. The contractions were unobtainable or weak following an ischemia of 2 hours and could not be elicited after longer periods. When this absence of

TABLE II

The Time of Appearance of Individualization of Fibers and Abnormal Disks, Correlated with Disappearance of Contractility, in the Tibialis Anticus of 25 Rabbits

Duration of ischemia	No. of animals	Individualization		Abnormal anisotropic disks		Contractible muscles
		No. of animals affected	Fibers	No. of animals affected	Fibers	
(*hours*)			(*per cent*)		(*per cent*)	
2	4	0	0	0	0	4
4	4	4	60	2	10	1
6	4	4	100	4	42	0
12	4	4	100	4	60	0
18	2	2	100	2	70	0
24	4	4	100	4	90	0
48–96	3	3	100	3	90	0

contractile response was correlated with the type of lesion it was apparent that it first became absolute at about the time of, or just prior to, the appearance of the abnormal cross striations. The association was that loss of function preceded the formation of the disks, whereas the presence of disks invariably indicated a loss or depression of function dependent upon the extent of the change.

TABLE III

The Time and Extent of Occurrence of Individualization of Muscle Fibers and Abnormal Cross Striations in Ischemic Tibialis Anticus of 14 Rats

Duration of ischemia	No. of animals	Individualization of fibers	Abnormal anisotropic disks	Contractible muscles
(*hours*)				
1	3	0	0	3
2	4	4	0	2
4	3	3	3	0
8	4	4	4	0

In a series of 14 rats in which the blood supply was permitted to return to the limb after varying periods of ischemia, this relationship was apparent. The muscles deprived of blood for an interval less than 12 hours contained a few small areas of normal syncytoid tissue, commingled sparsely with the numerous abnormal fibers composed of disks. These muscles were only weakly contractile. The muscle ischemic for 12 hours never regained its function and was composed completely of abnormal fibers. It is noteworthy that even after an ischemia of 3

hours the return of blood flow did not prevent the further extensive development of abnormal fibers.

During the early stages of cytoplasmic change no alteration in the nucleus was detectable.. Normally the nucleus is of a diffuse, hazy, bluish gray (hematoxylin and eosin) with a few discrete, ill defined clumps of chromatin. After continuous ischemia for 12 or more hours the haziness vanished and the chromatin clumps were more conspicuous and numerous, leaving a clear structureless nucleoplasm. After 24 hours the nuclear membrane faded until eventually it completely disappeared through karyolysis with disruption of the entire structure.

Discussion

In freshly fixed rabbit, rat, and human skeletal muscle a compactness of structure which imparted a syncytium-like appearance was observed. This structural arrangement of closely adherent fibers in skeletal muscle has led certain observers [17-19] to consider it as syncytial, like cardiac muscle tissue. However, I am not inclined to accept the reasoning that either a syncytium-like appearance or difficulty in teasing the fibers apart warrants the designation of this tissue as a true syncytium, because the ease and completeness with which the fibers separate in the earliest stage of ischemia strongly indicate that the union between adjacent fibers is not intimate nor tenacious. However, owing to its constancy in normal muscle, this syncytium-like structure may be regarded as an important histological feature of healthy muscle, which has been designated as "syncytoid." This feature, together with the scarcity and vagueness of cross striations which Leser [2] observed and the accentuation of longitudinal striations, affords a picture strikingly different from that usually drawn by histologists, who accentuate the cross striations. Schäfer [20] did remark, however, that longitudinal striation is seen better in proportion as cross striation is less marked. Millar [21] advocated that for the better demonstration of the structure of skeletal muscle it is preferable to use tissue which has been cooled overnight at ice box temperature, because otherwise the cross striations are vague and interfered with by longitudinal striation. Mallory [22] emphatically stated that to demonstrate the "myoglia fibers" autopsy tissue is practically useless and urged the use of muscle tissue obtained at operation. This inverse proportion of longitudinal and cross striations, with the enhancement of one at the expense of the other, tends to substantiate Carey's [23] concept of the entire muscle fiber as a single morphological and functional unit composed of an undifferentiated "neuromyoplasm." Certainly the reciprocal evanescence of both structures does not support either as the sole permanent structural unit.

The considerable importance attached to the mere presence of cross striations as an indication that a particular fiber or group of fibers is normal requires modification. The finding that, under the conditions of acute and chronic ischemia, the cross striations are rendered conspicuous and coarse, and are altered in type, suggests not only that cross striations do not always represent viable muscle but that they may, if of a certain type, actually signalize nonviable, degenerated fibers. This type of peculiar discoid cross striation was first described by Bowman,[24] although he believed it to be a normal feature; such cross striations have been referred to as Bowman's disks. They have been demonstrated by Leser [2] and photographed by Clark,[14] under circumstances which leave no doubt of their degenerative nature; Clark named them "conchoidal plates," and found them in degenerated muscle grafts 7 and 11 days old. Clarke [8] observed them in muscle tissue from cases of clinical and experimental Volkmann's contracture, of 70, 97, and 300 days' duration. The significance of the disks has usually been overlooked; for example, among Griffiths' [7] illustrations of ischemic contracture one shows these peculiar cross striations which he simply referred to as "preservation of striations," as have other observers including Wells.[10] In an illustration of Fishback and Fishback [9] where an absence of striations is indicated, these disks are conspicuous.

I have used the tibialis anticus to determine the time and rate of appearance of these changes. This muscle invariably underwent massive necrosis when ischemia was sufficiently prolonged. Although Wilson [6] indicated that the quadriceps was most frequently involved in his cases, he based his figures on scattered foci of necrosis and excluded muscles with extensive necrosis, so that his criteria were different. When necrosis occurred in other muscles in my series the rate of appearance and character of the lesions paralleled closely those found in the tibialis anticus.

In numerous sections of normal muscle from a variety of species, including man, rabbit, and rat, I have been unable to find Bowman's disks. On the contrary, they are a constant feature in all of my examples of *ischemic* muscle, with a constancy of time of appearance and rate of development. Since they are coincident in time of appearance with biochemical irreversibility in ischemic muscle,[25] they may represent the morphological manifestation of structural irreversibility consequent upon the establishment of static biochemical equilibrium or death due to ischemia. According to Schoenheimer,[26] in order to maintain structure against its tendency to collapse energy must be expended continuously. Under circumstances in which the oxidation, phosphorylation, and synthetic processes are known to cease, *e.g.*, ischemia,

their cessation is closely followed by such structural collapse, which is seen, in this instance, as a peculiar aggregation of the cytoplasmic constituents.

It is averred by Clark [14] that the formation of these disks is induced by formaldehyde used as a fixative, which cross links such chain molecules of myosin as may be in a more parallel arrangement. Against this it may be cited that Bowman [24] first described these structures 53 years before formaldehyde was introduced into cytological technic.[27] Furthermore, I have demonstrated them with fixatives other than formaldehyde, e.g., Zenker's fluid and alcohol, and in freshly teased ischemic muscle. Moreover, Fischer [28] found that 40 per cent formaldehyde has only slight power of precipitation of proteins and a 4 per cent solution none whatever; on the contrary it renders protein solutions incoagulable by many coagulating agents. It may be pointed out in addition that if formalin alone produced this alteration it should be seen in the normal formaldehyde-fixed muscle, where it was never found. Nevertheless, it is not improbable that the fixative acts upon some fundamentally altered protein, which, since it comprises most of the muscle protein, is most likely myosin. The manner of action postulated by Gustavson [29] is that formaldehyde effects methylene cross links between peptide chains and micellar units; this view is shared by Theis and Ottens [30] and by Wilson.[31] Since this conclusion is reached from study of the collagen type of protein molecule and not the myosin-keratin type, and since the detailed structure of protein molecules is still largely unknown,[32] as is also the exact mode of union of formaldehyde with them, it would be hazardous to attribute such a change in the protein of ischemic muscle solely by analogy to altered or new chemical linkages. Furthermore, with the recognized importance of "coacervats" [33] in biological phenomena, their state in all systems reaching the equilibrium of death requires consideration. That alteration in colloidal state may constitute the basis of these changes is indicated by Bechhold,[34] who may be quoted:

"*With the occurrence of death protoplasm gelatinizes,* Brownian movement of the smaller particles ceases, and the structure of the gel appears in the ultramicroscope as a conglomeration of many reflecting platelets. It makes a substantial difference whether the protoplasm slowly dies or is suddenly killed by a fixative (alcohol, formalin, etc.). In the first instance there is a precipitation (flocculation), whereas, in the latter there is a *stiffening;* this difference may be readily recognized under the ultramicroscope."

Whatever may be the nature of the underlying change, its importance as a morphological manifestation appears to be that it represents a terminal structural event in the process of cell death. Under restricted

conditions of ischemia it is possible to enumerate this series of events as cessation of the processes of oxidation and phosphorylation, depletion of biochemical reserves, loss of contractility, biochemical irreversibility, and finally irreversible structural change or degeneration.

SUMMARY AND CONCLUSIONS

1. Skeletal muscles of rabbits and rats, rendered ischemic for periods of from 1 to 96 hours by ligation of vessels and application of tourniquets, were studied histologically and compared with normal contralateral muscles.

2. The characteristics of normal, rapidly fixed muscles are a syncytoid structure with vague cross striations and a conspicuousness of undulant longitudinal striations. Nuclei are deeply basophilic, with a fine chromatic network.

3. With ischemia of 2 to 4 hours' duration the fibers are individualized, longitudinal striations disappear, and cross striations become a conspicuous cytological feature. After longer periods of ischemia abnormal anisotropic disks, Bowman's disks or conchoidal plates, appear and involve the muscle fibers in increasing numbers up to 18 hours of ischemia, at which time they are nearly ubiquitous. They are true degenerative forms and not artefacts caused by fixation and sectioning.

4. Weakness or absence of contractility precedes and accompanies the appearance of these disks and is correlated with their presence and extent of involvement, so that they serve as a clear indication of nonviable fibers and constitute a morphological manifestation of cell death in skeletal muscle.

REFERENCES

1. Wells, H. G. Chemical Pathology. W. B. Saunders Co., Philadelphia, 1925, ed. 5, pp. 65–90.
2. Leser, E. Untersuchungen über ischaemische Muskellähmungen und Muskelcontracturen. *Samml. klin. Vortr.*, 1884, Chir. no. 77, 2087–2114.
3. Brooks, B. Pathologic changes in muscle as a result of disturbances of circulation. An experimental study of Volkmann's ischemic paralysis. *Arch. Surg.*, 1922, 5, 188–216.
4. Jepson, P. N. Ischaemic contracture. *Ann Surg.*, 1926, 84, 785–795.
5. Middleton, D. S. The pathology of congenital torticollis. *Brit. J. Surg.*, 1930–31, 18, 188–204.
6. Wilson, W. C. Occlusion of the main artery and main vein of a limb. *Brit. J. Surg.*, 1933, 20, 393–411.
7. Griffiths, D. L. Volkmann's ischaemic contracture. *Brit. J. Surg.*, 1940–41, 28, 239–260.
8. Clarke, W. T. Volkmann's ischaemic contracture. *Canad. M. A. J.*, 1946, 54, 339–341.

9. Fishback, D. K., and Fishback, H. R. Studies of experimental muscle degeneration. I. Factors in the production of muscle degeneration. *Am. J. Path.*, 1932, 8, 193–209. II. Standard method of causation of degeneration, and repair of the injured muscle. *Am. J. Path.*, 1932, 8, 211–217.

10. Wells, H. G. The pathogenesis of waxy degeneration of striated muscles (Zenker's degeneration). *J. Exper. Med.*, 1909, 11, 1–9.

11. Stemmler, W. Die wachsartige Degeneration der Muskulatur bei infektionskrankheiten. *Virchows Arch f. path. Anat.*, 1914, 216, 57–77.

12. Forbus, W. D. Pathologic changes in voluntary muscle. I. Degeneration and regeneration of the rectus abdominis in pneumonia. *Arch. Path.*, 1926, 2, 318–339. II. Experimental study of degeneration and regeneration of striated muscle with vital stains. *Arch. Path.*, 1926, 2, 487–499.

13. Wolbach, S. B., and Frothingham, C. The influenza epidemic at Camp Devens in 1918. A study of the pathology of the fatal cases. *Arch. Int. Med.*, 1923, 32, 571–600.

14. Clark, W. E. L. An experimental study of the regeneration of mammalian striped muscle. *J. Anat.*, 1946, 80, 24–36.

15. Olovson, T. Beitrag zur Kenntnis der Verbindungen zwischen A. ilica interna und A. femoralis beim Menschen nebst tierexperimentellen Studien über die Morphologie des Kollateralkreislaufs nach Unterbindung der A. ilica externa und A. femoralis. *Acta chir. Scandinav.*, 1941, suppl. 67, 86, 1–216.

16. Clark, W. E. L., and Blomfield, L. B. The efficiency of intramuscular anastomoses, with observations on the regeneration of devascularized muscle. *J. Anat.*, 1945, 79, 15–32.

17. Jordon, H. E. Textbook of Histology. D. Appleton-Century Co., New York, 1934, ed. 6, p. 95.

18. Huber, G. C. On the form and arrangement in fasciculi of striated voluntary muscle fibers. A preliminary report. *Anat. Rec.*, 1916–17, 11, 149–168.

19. Godlewski, E. Die Entwicklung der Skelett- und Herzmuskelgewebes der Säugethiere. *Arch. f. mikr. Anat.*, 1902, 60, 111–156.

20. Schäfer, E. A. A Course of Practical Histology. Henry C. Lea, Philadelphia, 1877, p. 115.

21. Millar, W. G. Observations on striated muscle. *J. Path. & Bact.*, 1933, 37, 127–135.

22. Mallory, F. B. Pathological Technique. W. B. Saunders Co., Philadelphia, 1938, p. 177.

23. Carey, E. B., Massopust, L. C., Zeit, W., and Haushalter, E. Studies on ameboid motion and secretion of motor end-plates. VII. Experimental pathology of the secretory mechanism of motor end-plates in thermal shock. *Am. J. Path.*, 1946, 22, 175–233.

24. Bowman, W. On the minute structure and movements of voluntary muscle. *Phil. Tr. Lond.*, 1840, 130, Pt. II, 457–501.

25. LePage, G. A. Biological energy transformations during shock as shown by tissue analyses. *Am. J. Physiol.*, 1946, 146, 267–281.

26. Schoenheimer, R. The Dynamic State of Body Constituents. Harvard University Press, Cambridge, 1942, pp. 63–65.

27. Blum, F. Der Formaldehyde als Härtungsmittel. *Ztschr. f. wissensch. Mikr.*, 1893, 10, 314–315.

28. Fischer, A. Fixierung, Färbung und Bau des Protoplasmas. G. Fischer, Jena, 1899, p. 24.

29. Gustavson, K. H. Formaldehydens reaktion med proteiner. *Svensk Kem. Tid.*, 1940, **52**, 261–277.

30. Theis, E. R., and Ottens, E. F. Studies in aldehyde tannage. V. Effect of temperature, formaldehyde concentration upon formaldehyde fixation. *J. Am. Leather Chem. Assoc.*, 1941, **36**, 22–37.

31. Wilson, J. A. Four fundamental types of tannage. *J. Am. Leather Chem. Assoc.*, 1941, **36**, 590–602.

32. Astbury, W. T. The hydrogen bond in protein structure. *Trans. Faraday Soc.*, 1940, **36**, 871–880.

33. Bungenberg de Jong, H. G. La coacervation. Les coacervats et leur importance en biologie. Hermann et Cie., Paris, 1936.

34. Bechhold, H. Colloids in Biology and Medicine. (Tr. by J. G. M. Bullowa.) D. Van Nostrand Co., New York, 1919, p. 279.

[Illustrations follow]

DESCRIPTION OF PLATES

PLATE 89

FIG. 1. Normal, formalin-fixed skeletal muscle of the rabbit. The fibers are closely approximated and appear syncytial. Hematoxylin and eosin stain. × 70.

FIG. 2. Normal tibialis anticus muscle from the rabbit. The longitudinal striations are strongly evident. Cross striations are indistinct and fine. Fibers are not separate. Hematoxylin and eosin stain. × 300.

FIG. 3. Tibialis anticus of a rabbit after 4 hours of ischemia. The muscle fibers are individualized and separate. Hematoxylin and eosin stain. × 70.

FIG. 4. High power photomicrograph of the same muscle seen in Figure 3. The longitudinal striations are not seen clearly, but the cross striations stand out prominently, with well marked isotropic bands. Hematoxylin and eosin stain. × 300.

FIG. 5. Tibialis anticus of a rabbit after 6 hours of ischemia. The individualization is further accentuated. Contours of the fibers appear more cylindrical. Hematoxylin and eosin stain. × 70.

FIG. 6. High power view of the same muscle seen in Figure 5. The new type of broad anisotropic disk and the splitting off of these disks are notable features. Hematoxylin and eosin stain. × 300.

PLATE 90

Fig. 7. Tibialis anticus of a rabbit after 96 hours of ischemia. The fibers are broad, individualized and have developed multiple transverse cracks. Hematoxylin and eosin stain. × 70.

Fig. 8. High power view of the same muscle seen in Figure 7. Here the splitting off and isolation of disks, responsible for the transverse cracking, are seen. Hematoxylin and eosin stain. × 300.

Fig. 9. Zenker-fixed tibialis anticus of a rabbit after 96 hours of ischemia. The fixative accentuates the Bowman's disks. Hematoxylin and eosin stain. × 300.

Fig. 10. Extensor muscle (tibialis anticus) of a rat after 12 hours of ischemia. The alterations are similar to those in the rabbit. Tourniquet was released shortly before the muscle was excised. Hematoxylin and eosin stain. × 300.

Fig. 11. Tibialis anticus of a rat after 8 hours of ischemia. The fragmentation, disk formation, and cracking are evident. Hematoxylin and eosin stain. × 300.

Fig. 12. Normal human pectoralis major muscle, taken at autopsy within an hour of death. The pattern is identical with that of normal rabbit and rat muscle. The transverse striations are more conspicuous, however, in autopsy tissue. Hematoxylin and eosin stain. × 300.
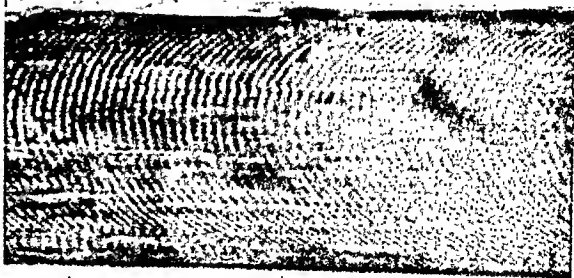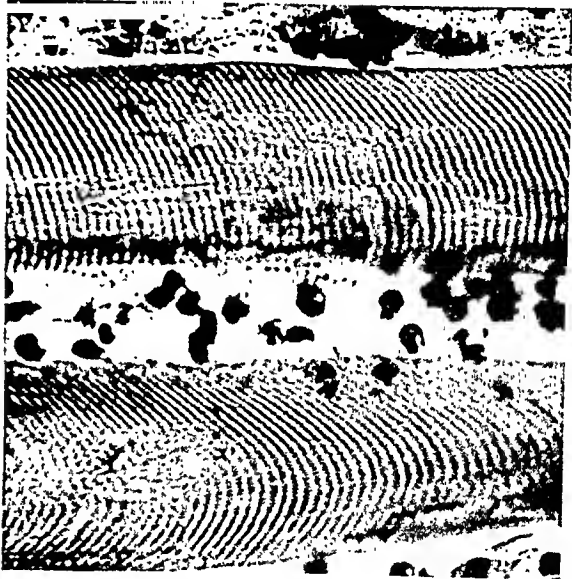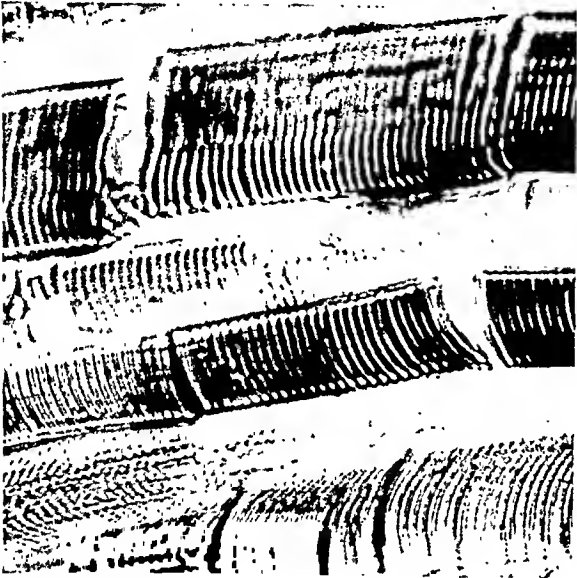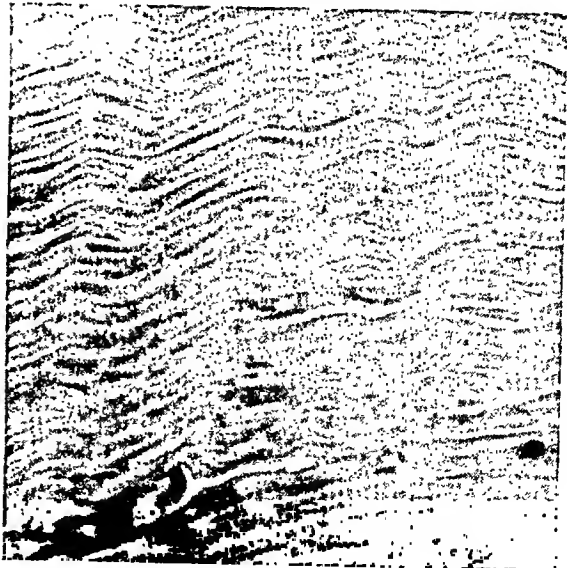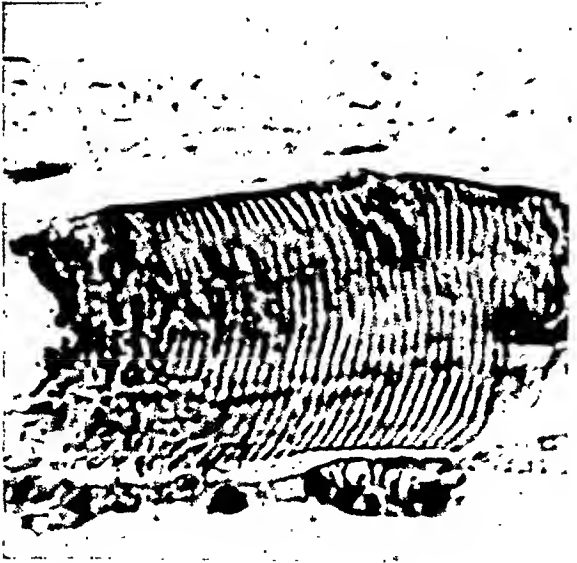
Harman

Skeletal Muscle in Ischemia

THE BENIGN GIANT CELL TUMOR OF TENDON SHEATHS

AN EXAMPLE OF SCLEROSING HEMANGIOMA *

LEE N. FOSTER, M.D.

(From the Department of Pathology, Harvard University Medical School, Boston 15, Mass.)

The classification of a tumor is usually based upon the morphologic characteristics of its numerically predominant cell, which reveal its relationship to some normal anatomic structure. This method, simple and direct, may fail completely if the correlation it demands is lost because of changes induced in the form of the predominant cell by neoplastic change.

An example of a lesion representative of this difficulty is the benign giant cell tumor of tendon sheaths. Referred to in the literature by various descriptive terms such as giant cell tumor, xanthoma, and tendon sheath myeloma, it is characterized by the formation of a slowly growing benign tumor, the histologic make-up of which consists of large numbers of multinucleated giant cells, certain lipid-laden cells, and frequent collections of macrophages containing hemosiderin pigment. These cellular components are supported by a dense stroma of fibroblasts and collagen. Many possible relationships are suggested by these numerous, seemingly individual cell types, and when only a single specimen is available for study, attempts to weave them into a unit process fail. When a series of tumors is reviewed, certain sequential tissue changes will be observed, thus presenting an alternative method of study by which the behavior of individual tissue elements may be tabulated for integration when sufficient factors are established. This approach has been used with success by Gross and Wolbach[1] in their study of sclerosing hemangiomas.

SUMMARY OF LITERATURE

Since the original description of the giant cell tumor by Broca in 1861 (cited by Heurtaux[2]), a considerable literature has accumulated.

Significant reviews have been published by Heurtaux,[2] Tourneux,[3] Bellamy,[4] Garrett,[5] Mason and Woolston,[6] and Galloway, Broders, and Ghormley,[7] the most comprehensive being the latter (1940), in which 339 cases were collected. In 1941, Jaffe, Lichtenstein, and Sutro[8] added 55 cases, but since then only occasional single case reports have appeared. Because of these numerous exhaustive surveys only a sum-

mary of the significant data pertaining to the giant cell tumor will be presented.

Earlier writers, stressing cellular pleomorphism, classed the growth with sarcomas, but as the weight of clinical evidence increased its benign nature became evident, a fact first realized by Heurtaux [2] in 1891. There then followed a brilliant attempt by Bellamy [4] in 1901 to interpret the tissue antecedent to the tumor. He listed as characteristic "giant cells, proliferating endothelium, and cells showing advanced stages of fatty degeneration" and concluded that the tumor was an endothelioma of angioblastic origin. Twelve years later Fleissig,[9] comparing the lesion to a granuloma, interjected the possible inflammatory origin of the tumor, an opinion shared at present by Jaffe and co-workers [8] who regard the "phagocytic property of the stromal cell and progressive collagenization and hyalinization" as fundamental. Recently, Thannhauser and Magendantz,[10] in their excellent study of xanthomatous diseases, included the tendon sheath lesion, cataloguing it under the heading of primary essential xanthomatosis. Although only two cases of xanthoma of tendon sheaths were presented in their paper, a more intensive study of 88 synovial tumors by Galloway and co-workers [7] resulted in agreement. Thus at present there are three different interpretations of the mode of origin of the tumor existent in the literature: one considering it a benign neoplasm, another an inflammatory process, while the third holds it to be a tissue manifestation of a disturbance in lipid metabolism.

MATERIALS AND METHODS

The material for this study was selected from the files of the Pathology Department of the Peter Bent Brigham Hospital and Rhode Island Hospital. One tumor obtained from the Pathology Department of the Beth Israel Hospital and two from the Lovett Fund Collection of the Department of Pathology of Harvard Medical School also were utilized. In this manner a total of 42 specimens diagnosed as benign giant cell tumor were chosen and the case records reviewed.

The tissues were fixed in Zenker's fluid or formalin, embedded in paraffin, and stained with hematoxylin and eosin, or eosin and methylene blue. On 30 of the specimens the following histologic technics were employed routinely: eosin and methylene blue, phosphotungstic acid hematoxylin, Mallory's aniline blue, Laidlaw's method for reticulum, and Turnbull's reaction for iron. Frozen sections of the available formalin-fixed material were stained with sudan IV, Nile blue sulfate, and scharlach R. Scharlach R was used in a diacetin (dihydroxyacetone) base. In a few of the specimens attempts were

made to demonstrate cholesterol in tissues by the technics of Windaus and of Golodetz and several unstained sections were examined under the polarizing microscope. Sections of one of the tumors were incinerated for ash residue.

Summary of Clinical Material

The clinical information available on these patients recapitulated that recorded in the literature. The tumors were painless growths causing no subjective symptoms, arising more frequently in women than in men. The ages of the patients in this series varied from 17 to 68 years, while the stated duration of the lesions ranged from 3 months to 15 years, averaging 3.8 years. The point of origin was usually in the tendon sheaths of the fingers and the majority of tumors were described by the surgeons as encapsulated, often mentioned as "shelling out" easily, although two of the growths were noted to be attached to the periosteum. Three of the 42 tumors of this series were recurrences.

Gross Appearance

Grossly, the benign giant cell tumor is a lobulated, seemingly encapsulated growth, seldom measuring more than 3 cm. in its greatest diameter. It has a firm, rubbery consistency and cuts with a tough resistance to the knife. The cut surface is dull yellow, streaked with gray trabeculae, and at its periphery a slender, brilliant orange-yellow line often parallels the border of the tumor.

Microscopic Description

In studying the tumors many unusual, enormously pleomorphic cell pictures were encountered which, despite the finding of transitional forms between the various cells, made interpretation difficult or impossible from the perspective of cell type alone. A clearer concept of the tumor process was gained when the behavior of the cellular elements was correlated with the stromal overgrowth that took place relatively rapidly in all of these specimens. In this manner the effect of sclerosis on the tumor cells might be observed and the resultant sequential changes in the tissue patterns better understood.

Those specimens in which little fibrosis was evident had a highly vascular structure composed of both arteriolar and capillary vessels, arborizing in the usual manner and separated from one another by a cellular tissue matrix. The blood vessels, frequently containing erythrocytes, were lined with elongated, oval, swollen, endothelial cells, the nuclei of which, when stained with hematoxylin, were pale dusty blue and contained some scattered chromatin material enclosed in a distinct

nuclear membrane. A prominent nucleolus was always present. Fibroblasts, occurring in greatest frequency about the larger vessels, gave off fibrils of collagen to intermingle with occasional smooth muscle cells, completing the formation of the blood vessel wall.

The cells of the intervascular tissue possessed nuclei strikingly similar to those of the endothelial cell. They were oval to round, slightly irregular, assumed a pale basophilic stain, and contained one or two distinct nucleoli. Mitotic figures were few. The plentiful cytoplasmic substance might be evenly basophilic at times but more often tended to contain clear vacuoles of lipid. Many of those cells, the cytoplasm of which stained with basic dyes, might be seen to be filled with fine droplets of lipid when stained with scharlach R in diacetin, although at this point it should be emphasized that no lipid was demonstrable in those regions of the tumor where active proliferation of these cells was taking place. When the fatty substance was abundant it was easily stained with sudan IV or Nile blue sulfate. The intervascular cells were separated from one another by a delicate lacework of collagen in which a fine reticulum was present.

Stromal overgrowth, beginning in multiple foci, was made manifest in its earliest phases by increased deposition of collagen and reticulum about the smaller blood vessels. These accumulations increased and coalesced, extending along the walls of capillaries, and constricting and usually obliterating vascular lumina, while fibroblasts reached through the tissue matrix to form a latticework of collagen segregating intervascular cells into irregular clusters. Eventually this process resulted in massive aggregates of hyalinized collagen in which little or no cellular detail was recognizable.

The collagen thus formed was stained less brilliantly by specific stains than the surrounding tendon sheath. Its structure might be fibrillar but more frequently had a glassy, homogeneous, hyaline appearance. In it, reticulum fibrils, as demonstrated by silver impregnation methods, were delicate and plentiful but elastic fibers were few. Micro-incineration revealed nothing characteristic in its mineral content.

The sclerosing process had a pronounced effect upon both the organoid and the cellular structure of the tumor. Endothelial cells thus encased in a firm sheath of collagen continued to proliferate as the fibrous tissue about them increased. As blood vessel lumina became occluded, the usual vascular arrangement of the tumor was disrupted, in some specimens to such an extent that poorly defined vascular channels opened through masses of intervascular cells. In such instances the cells in immediate relationship to such spaces usually contained large amounts of hemosiderin.

As the endothelial and intervascular cells were enclosed in groups by the sclerosing process, many coalesced to form multinucleated giant cells while others retained their individual character separated from one another by a delicate reticulum. The size of the latter cells tended to increase, and as their nuclear volume enlarged disproportionately their cytoplasm became relatively sparse but still might maintain some of its lipid content. Occasional mitotic figures occurred in these cells, showing that despite the regressive tendencies induced by the accumulating stroma, the process of sclerosis in itself was not sufficient to inhibit growth.

The configuration of the giant cell might vary from a simple rounded mass of cytoplasm containing a few nuclei to a branching stellate, syncytium in which hundreds of nuclei were present. The nuclei were somewhat smaller, but had staining characteristics essentially similar to those of the intervascular cell. They were uniformly oval, having no special arrangement except in certain instances when the giant cell appeared to be in the process of fusing with an intervascular cell, in which case they assumed a polar position opposite to that of the single cell. Mitotic figures were seen rarely. The cytoplasm of these cells had a basophilic, smooth ground-glass appearance. Frequently this uniformity was interrupted by small, clear vacuoles of lipid, or golden brown granules of hemosiderin, and often intervascular cells were found completely engulfed in the cytoplasm of the giant cell.

Deposits of hemosiderin occurred in close correlation with the onset of the sclerosing process, in some instances so precisely as to define a focus of sclerosis as clearly as a specific collagen stain. As the fibroblastic stroma began to increase, fine granules of refractile golden brown pigment appeared in the cytoplasm of the intervascular cells and endothelial cells and, to a lesser extent, in the intercellular substance. When stained by Turnbull's method, the pigment granules stained deep blue, demonstrating their iron content.

As the continuity of the vascular bed of the tumor was interrupted, entrapped erythrocytes were engulfed by surrounding endothelial and intervascular cells. The degenerating red cells became swollen, lost their regular outline, and assumed a granular appearance as they were phagocytosed. The eventual disposition of the pigment probably proceeded in its usual manner, by hemoglobin breaking down to hematoidin and the latter going into solution in tissues, as none of the specimens examined showed any tendency to maintain collections of hemosiderin once the sclerosing process was well advanced.

The tumor grew in a lobular manner, dissecting through the tendon sheath and breaking it up into slender septa that penetrated varying distances into the tumor, often so far as to divide one lobule from

another. Although overwhelmed by sclerosis in its central portion, a lobule might exhibit evidence of growth at its periphery. In some specimens numerous mitotic figures might be seen in the intervascular cells as they proliferated in broad masses, pushing into the surrounding tendon sheath. Capillaries invaded the tendon sheath directly, only to become rapidly obliterated, leaving linear seams of hemosiderin and/or lipid-containing endothelial cells that continued to grow after their vascular structure had been destroyed. In consequence of this activity dense aggregates of hemosiderin appeared at the surface of the tumor and in the surrounding tendon sheath.

Although these deposits were rather sparse in 12 specimens, they were present in all of the 42 tumors examined.

When the lipophagic activity of the intervascular cell became extreme, the cell assumed a characteristic appearance to which the terms foam cell and xanthoma cell have been applied. In 22 of these tumors this tendency was not pronounced, but in the other specimens "xanthoma cells" were arranged in collar-like sheets at the far periphery of the tumor and rather abruptly differentiated from it. Less frequently they were scattered diffusely through its substance. When this arrangement was seen it was noted that the sclerosing process had failed to obliterate vascular lumina even though many of the blood vessel walls were composed of dense hyaline collagen. Neither was there any evidence of destruction of the vascular network in the solid masses of foam cells.

As seen in hematoxylin and eosin preparations, the xanthoma cell possessed an abundant foamy cytoplasm composed of small, evenly sized, clear vacuoles separated from one another by a delicate pale basophilic, granular lacework. When treated with sudan IV, Nile blue sulfate, or scharlach R, this cytoplasmic material colored brilliantly, demonstrating its lipid nature. The reticular background appeared pale blue when stained by Mallory's aniline blue method for collagen.

Chemical analyses of these tumors [7,10] showed some of them to contain large amounts of cholesterol. Utilization of the available histochemical methods for its demonstration, such as the digitonin reaction and the procedures of Liebermann-Burchardt and of Golodetz, yielded inconstant results. However, the Golodetz test was often positive, staining the foam cell a pale brown. When examined polariscopically the lipid appeared in the form of needle-like crystals, but lost its refractile qualities when the slide was warmed gently. As the tissue cooled after such treatment, many doubly refractile droplets appeared, suggesting the presence of cholesterol esters. It is thought that the needle-like forms observed represent crystalline fatty acid.

Although these lipophagic cells may be multinucleated, this feature was not pronounced as they seldom contained more than two or three nuclei. More characteristic of their behavior was the tendency of the nuclear chromatin to be.condensed and the nuclear membrane wrinkled, so that the nucleus became small, shrunken, and pyknotic, lying in a large mass of foamy lipid, eventually fragmenting and disappearing completely. In such instances focal collections of lipid might remain in the tissue in an extracellular position. The lipophagic activity of the intervascular cell, manifest shortly after cell proliferation ceased, remained as a continuous function impaired only by stromal overgrowth until the death of the cell.

Although the precise nature and origin of the lipid is not understood, it is apparently derived from the blood stream and consists of a mixture of cholesterol esters and neutral fats. These substances are definitely associated with degenerative phenomena exhibited by the intervascular cell.

DISCUSSION

As the giant cell tumor grows, it infiltrates the surrounding tendon sheath from which its capsular structure is derived. Transitional forms between the giant cell, intervascular cell, and foam cell indicate them to be morphologic variations of the same type cell, the cytologic characteristics of which are closely correlated to the stromal reaction. As stromal overgrowth proceeds and the vascular networks of the tumor are interrupted, the intervascular and endothelial cells phagocytose hemosiderin liberated from fragmented erythrocytes. Many of the tumor cells coalesce to form multinucleate giant cells. If blood vessel lumina are not obliterated by sclerosis, the intervascular cells accumulate lipid until the amounts of fatty substance become so great that the cells fragment and die. The proliferation of tumor cells is continuous, limited only by their own degenerative tendencies. Even in specimens in which sclerosis is far advanced, occasional mitotic figures occur in nests of tumor cells segregated by massive amounts of collagen. These lesions, enlarging progressively, restrained only by their own growth characteristics and characterized by a type cell occupying a definite and predictable relationship to its stroma, can be considered only as neoplasms.

The extreme vascularity of specimens uninvolved by sclerosis immediately suggests their angiomatous nature. This detail is especially well demonstrated in sections impregnated with silver by Laidlaw's method and counterstained with van Gieson's connective tissue stain as advised by Stout.[11] Proliferating sheets of intervascular cells and infiltrating capillaries seen at the surface of the tumor are integral fea-

tures of its growth, while the lipid accumulations, stromal overgrowth, and hemosiderin deposition are phases of a regressive process. These tissue reactions are manifestations of the activity of various components of the vascular system and are centered about the blood vessel pattern of the tumor. The behavior of the tumor cells, as well as the anatomic structure of specimens, uninvolved by sclerosis, define the tumor as a sclerosing hemangioma.

Opinion regarding this process as a metabolic disturbance was not confirmed by this study. Although the foam cell has been thought to be the anatomic expression of a disturbed blood lipid stoichiometry, fat, appearing in these cells secondarily, represents a degenerative phenomenon that is seen in numerous other neoplastic and inflammatory diseases.[12] Proliferation of tumor cells occurs before lipophagocytosis is demonstrable and ceases as large amounts of lipid appear in the cytoplasm of the intervascular cell.

It should be mentioned that the sclerosing hemangiomas are to be separated from those infiltrates which are definitely a part of the metabolic disturbances. Such lesions have a familial incidence and often appear in younger persons. They have been described [10,12] involving multiple tendon structures and other organs. Further investigation of this problem is warranted as the histologic description of certain of these specimens [7,10] does not coincide precisely with that of the sclerosing hemangioma.

Some authors, emphasizing the continuous increase of fibrous tissue, the phagocytic properties of the intervascular cell, and the giant cells seen in these lesions, have presumed them to be inflammatory in origin. If progressive fibrosis is to be considered a phase of an inflammatory reaction, its mechanism should be explained in a study of the stromal tissue sequences; but fibroblastic activity in these tumors is continuous, related to other cellular elements only through the effects it produces. Neither is it permissible to credit the phagocytic activity of the intervascular cell as indicative of inflammation. These items taken separately merely catalogue various cell functions but do not describe any process, as they do not integrate into a common pattern.

This tumor is believed to be the tendon sheath equivalent of the cutaneous sclerosing hemangioma described by Wolbach [13] in 1913 and more fully by Gross and Wolbach.[1] Even though minor differences between the two exist, such as a more uniform tendency toward phagocytosis of lipids and toward giant cell formation in the tendon sheath lesion, it must be remembered that these are quantitative differences, whereas the tissue sequences so clearly traced by Wolbach in cutaneous tumors are identical in both neoplasms. The sclerosing hemangiomas

of the central nervous system reported by Bailey and Ford [14] in 1942 are also identified with this group by those authors. Here again, quantitative differences are evident while tissue sequences remain the same. The stromal overgrowth occurring in tumors of the central nervous system is peculiar in that both glial and fibroblastic elements participate, but stromal behavior as well as the tumor cell components follow the patterns observed in sclerosing hemangiomas elsewhere.

Although a precise explanation of the mechanism of sclerosis was not found, it was noted in specimens of 1 year or less in duration that only minimal amounts of stromal overgrowth had occurred, an observation in conformity with another in the literature.[8] Neither was sclerosis pronounced in the peripheral portions of certain tumors in which growth, as evidenced by frequent mitotic figures in the intervascular cells, was rapid. Stromal overgrowth could not be correlated with the extent of degeneration of the intervascular cells but rather followed a slowly progressive course, as if representing an imbalance in the growth relationship between tumor cells and stroma.

The tendency of the tumor to recur, a feature hardly to be considered exemplary of a completely benign, slowly growing neoplasm, is well recognized [7,8] and three such instances are recorded in this series. Some explanation of this may be found when the tumor-tendon sheath interface is studied. Here it is seen that the portion of the tendon sheath forming the tumor capsule may be infiltrated to a considerable extent with capillaries and cell masses reaching from the surface of the neoplasm. Even though the main body of the tumor may shell out easily, fragments of actively growing neoplastic tissue may be left behind. For this reason care should be taken to extirpate the lesion widely.

The giant cell tumors occurring in the synovial structures of joint cavities and often discussed with the tendon sheath lesion as its synovial equivalent [7,8] are not included in this series. Although it is believed that such an identity exists, it is felt that the clinical and pathologic characteristics of the synovial lesion are sufficiently different to warrant separate discussion.

CONCLUSION

In its earliest phases of growth the benign giant cell tumor has a highly vascular structure that becomes disrupted by sclerosis, but proliferation of the intervascular and endothelial cells of the tumor continues, limited only by the degenerative tendencies inherent in these cells and to some extent by stromal overgrowth. These are features of autonomous growth and definitive of a true neoplasm.

As the development of the tumor progresses, certain of its cellular elements phagocytose lipid and hemosiderin while some coalesce with one another to form multinucleated giant cells. These activities represent behavior characteristics of the reticulo-endothelial system, and since they are centered about blood vessels it is concluded that the tumor is a sclerosing hemangioma.

REFERENCES

1. Gross, R. E., and Wolbach, S. B. Sclerosing hemangiomas. Their relationship to dermatofibroma, histiocytoma, xanthoma and to certain pigmented lesions of the skin. *Am. J. Path.*, 1943, 19, 533–551.
2. Heurtaux, A. Myélome des gaines tendineuses. *Arch. gén. de méd.*, 1891, 1, 40–54; 160–169.
3. Tourneux, J. P. Les sarcomes des gaines tendineuses. *Rev. de chir.*, 1913, 47, 817–854.
4. Bellamy, H. F. The myeloid tumour of tendon sheaths. *J. Path. & Bact.*, 1901, 7, 465–480.
5. Garrett, C. A. Tumors of the xanthoma type. *Arch. Surg.*, 1924, 8, 890–907.
6. Mason, M. L., and Woolston, W. H. Isolated giant cell xanthomatic tumors of the fingers and hand. *Arch. Surg.*, 1927, 15, 499–529.
7. Galloway, J. D. B., Broders, A. C., and Ghormley, R. K. Xanthoma of the tendon sheaths and synovial membranes. A clinical and pathologic study. *Arch. Surg.*, 1940, 40, 485–538.
8. Jaffe, H. L., Lichtenstein, L., and Sutro, C. J. Pigmented villonodular synovitis, bursitis and tenosynovitis. *Arch. Path.*, 1941, 31, 731–765.
9. Fleissig, J. Über die bisher als Riesenzellensarkome (Myelome) bezeichneten Granulationsgeschwülste der Sehnenscheiden. *Deutsche Ztschr. f. Chir.*, 1913, 122, 239–265.
10. Thannhauser, S. J., and Magendantz, H. The different clinical groups of xanthomatous diseases; a clinical physiological study of 22 cases. *Ann. Int. Med.*, 1937–38, 11, 1662–1746.
11. Stout, A. P. Hemangio-endothelioma: a tumor of blood vessels featuring vascular endothelial cells. *Ann. Surg.*, 1943, 118, 445–464.
12. Gruenfeld, G., and Seelig, M. G. The nature of so-called xanthoma. A critical review. *Arch. Path.*, 1934, 17, 546–573.
13. Wolbach, S. B. Hemangio-endotheliomata giving the histological appearances of "giant cell xanthoma." *Ztschr. f. Krebsforsch.*, 1912–13, 12, 440–441.
14. Bailey, O. T., and Ford, R. Sclerosing hemangiomas of the central nervous system. Progressive tissue changes in hemangioblastomas of the brain and in so-called angioblastic meningiomas. *Am. J. Path.*, 1942, 18, 1–27.

DESCRIPTION OF PLATES

PLATE 91

FIG. 1. Those specimens in which stromal overgrowth has not occurred have a highly vascular structure. Beginning perivascular accumulations of reticulum are seen. Laidlaw's method for reticulum. Van Gieson's counterstain. × 150.

FIG. 2. As sclerosis proceeds, the vascular structure becomes disrupted. Some increase in the size of the intervascular cells occurs. Laidlaw's method for reticulum. Van Gieson's counterstain. × 150.

1

2

Foster

Giant Cell Tumor of Tendon Sheaths
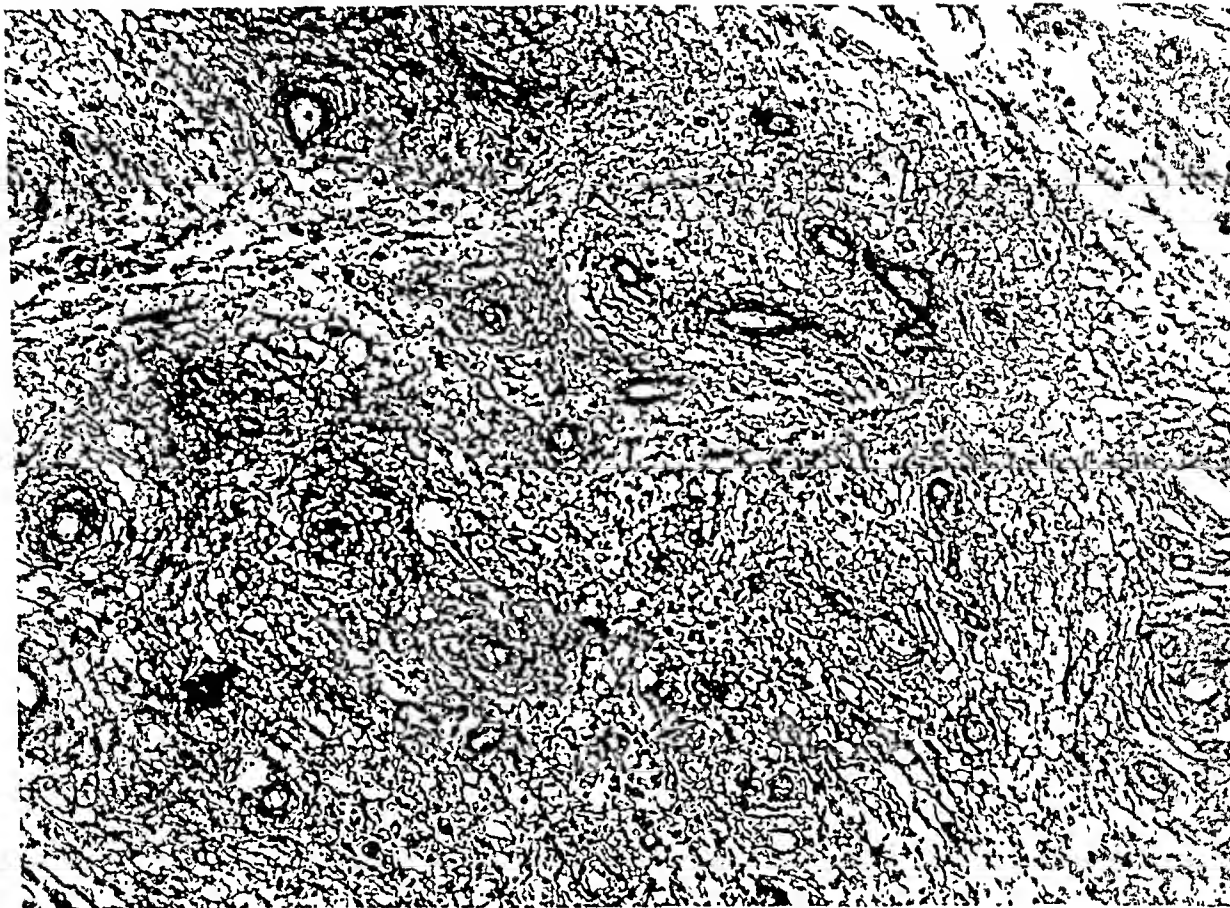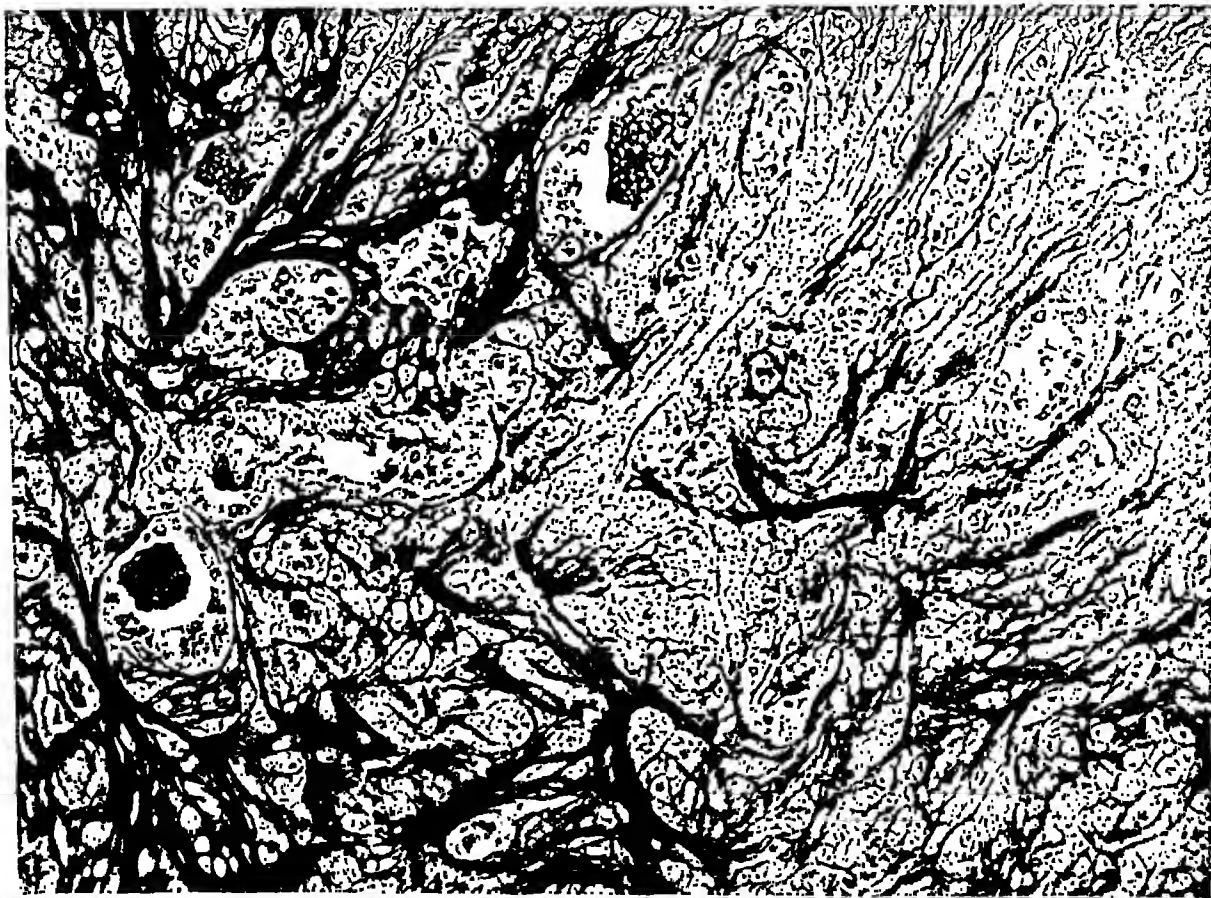
FIG. 3. Stromal overgrowth involving the central portions of two tumor lobules. A rounded solitary focus of sclerosis is present in the middle portion of the upper part of the section. Laidlaw's method for reticulum. Van Gieson's counterstain. × 10.

FIG. 4. Sequential section from the tumor illustrated in Figure 3 stained by Turnbull's reaction for iron. The solitary focus of sclerosis is especially well demarcated by the dark granules of iron pigment. × 10.

PLATE 92

3

4

PLATE 93

FIG. 5. As the collagenous stroma accumulates, intervascular cells coalesce to form multinucleated giant cells. Recently engulfed cells are plainly visible, represented by a nucleus surrounded by a clear ring of cytoplasm. Phosphotungstic acid and hematoxylin stain. × 150.

FIG. 6. A mitotic figure in a cord of endothelial cells. There are numerous granules of hemosiderin present. Eosin and methylene blue stain. × 500.

FIG. 7. The cellularity of the tumor becomes reduced by heavy deposits of collagen. Phosphotungstic acid and hematoxylin stain. × 300.

Foster

Giant Cell Tumor of Tendon Sheaths
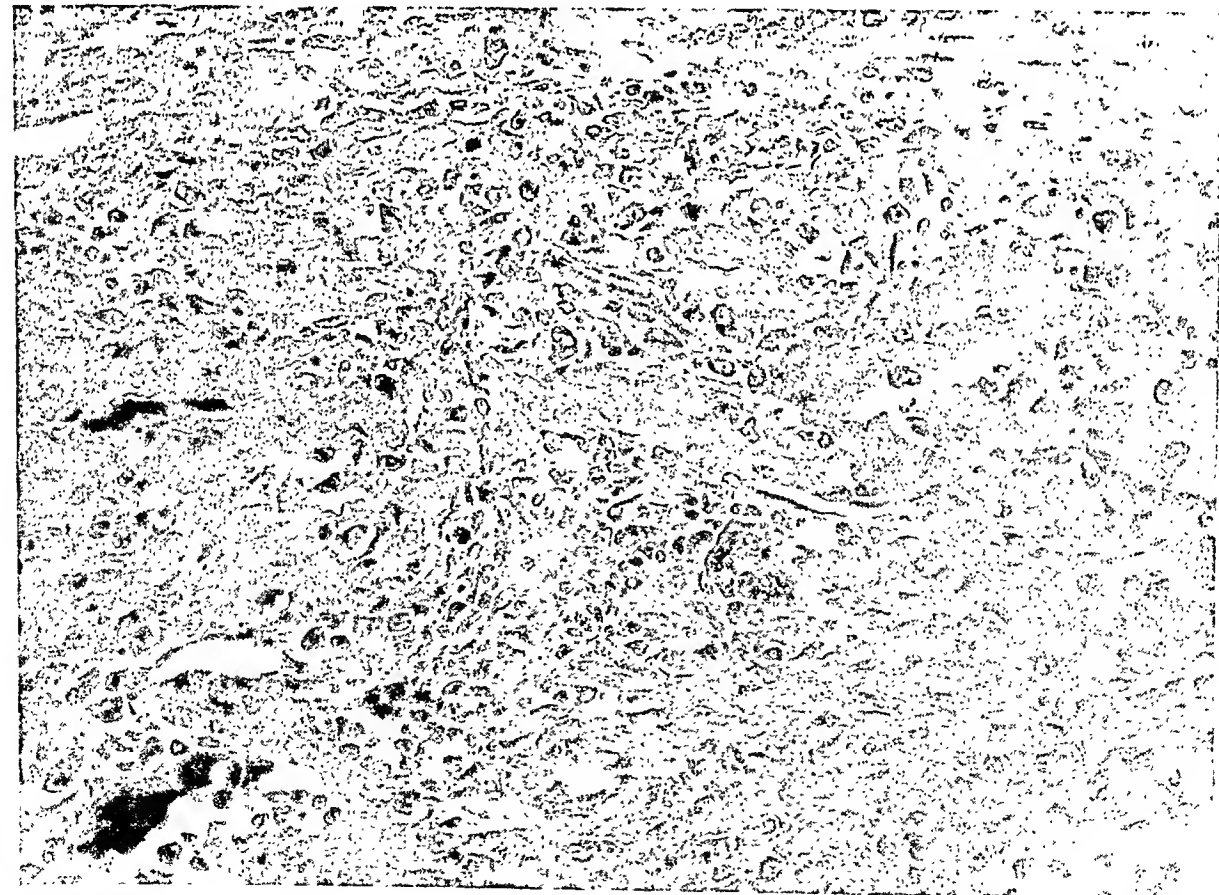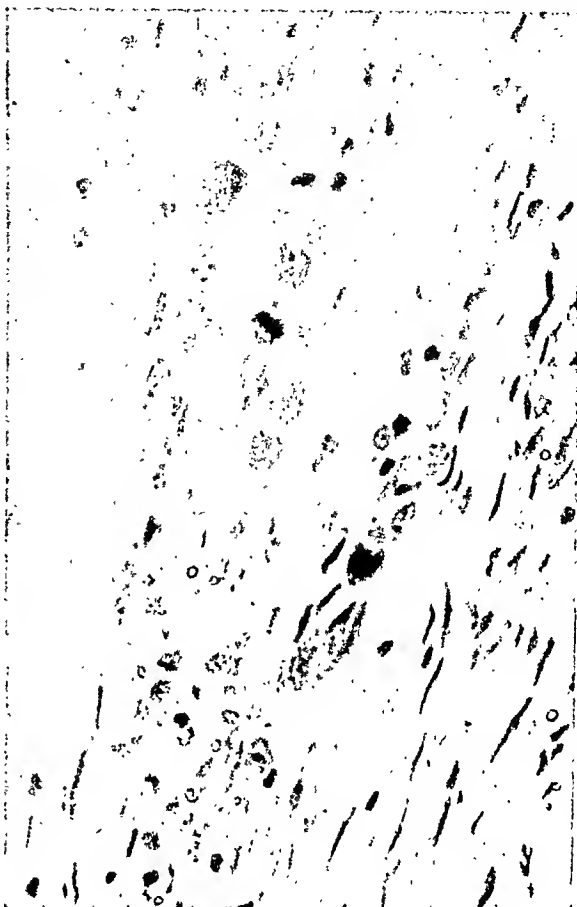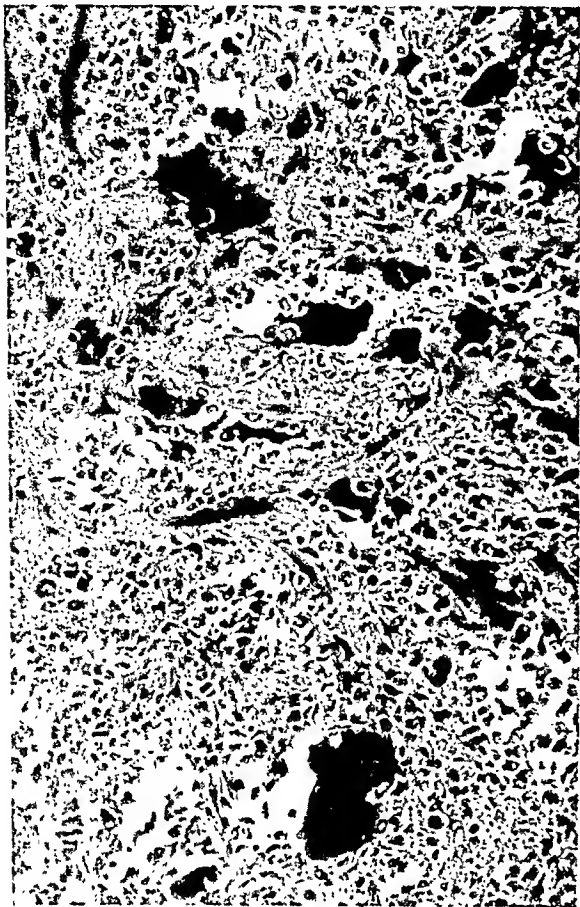
PLATE 94

FIG. 8. A mitotic figure in a nest of tumor cells segregated by the sclerosing process. Eosin and methylene blue stain. × 900.

FIG. 9. Birefringent crystals and globules of lipid seen under the polarizing microscope. The globules show the characteristic Maltese cross formation. × 150.

FIG. 10. Foam cells scattered through the tumor substance. In such instances continuity of the vascular bed of the tumor is left intact despite the heavy collars of collagen and reticulum surrounding the blood vessels. Mallory's aniline blue stain. × 150.

PLATE 94
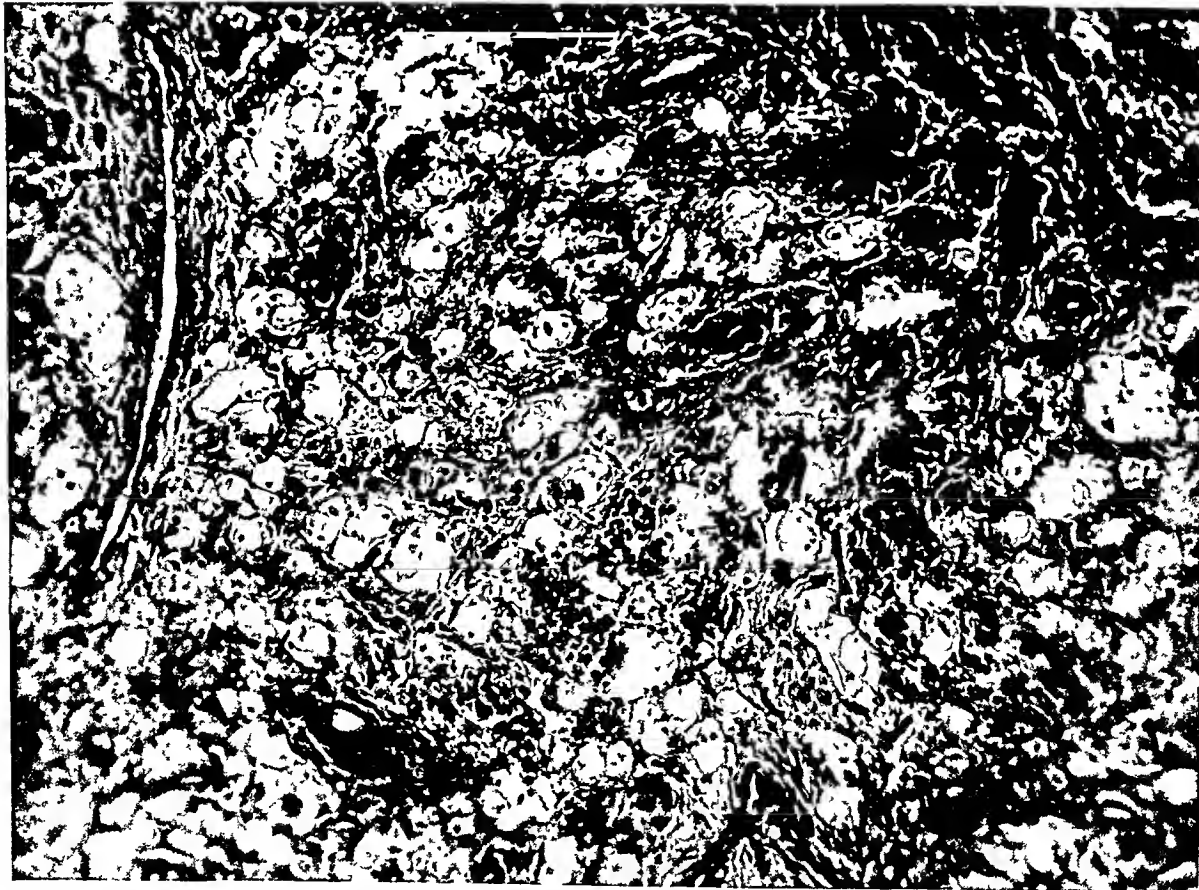
Foster

Giant Cell Tumor of Tendon Sheaths

ENDOTHELIAL-CELL SARCOMA OF LIVER FOLLOWING THOROTRAST INJECTIONS *

H. Edward MacMahon, M.D., Albert S. Murphy, M.D., and Margaret I. Bates. M.D.
(*From the Department of Pathology and Bacteriology, Tufts College Medical School, Boston, and the Cambridge Hospital, Cambridge, Mass.*)

As a pathological entity, a primary hemorrhagic endothelial-cell sarcoma of the liver is an unusual and interesting finding. When it is found in a patient who has received thorotrast, its presence may be of much greater significance. Thorotrast is colloidal thorium dioxide. It was first introduced into the practice of medicine 18 years ago when Blühbaum, Frik, and Kalkbrenner [1] described its use in roentgen visualization. As a diagnostic aid it has become widely adopted. It was first used in the field of gastroenterology, later in urology, and most recently in neurology. Its use has been condemned by some and extolled by others. Reeves and Stuck [2] have pointed out its disadvantages, and Yater [3] and McClure, Jankelson, and Osgood,[4] as recently as 1944, have championed its advantages.

REPORT OF CASE

The patient was a female who, at 40 years of age, had had a cholecystectomy. Ten years later she had experienced, over a 2-week period, attacks of paroxysmal pain under the costal margin. There was a history of considerable but vague abdominal distress during the subsequent 8 years. At the end of this period, when the patient was 58 years of age, a mass was described as filling the entire right half of the abdomen. A barium enema revealed displacement of the hepatic flexure and ascending colon to the left by a large mass in the right side of the abdomen. By pneumoperitoneum there were visualized one shadow filling the right half of the abdomen, interpreted as that of the liver, and a second shadow beneath the right diaphragm of undetermined origin. Repeated serological tests for syphilis at that time were strongly positive. Because of the uncertainty of the diagnosis a commercial preparation of thorotrast was given intravenously. Three daily doses of 25 cc. each were injected and very satisfactory contrast films were obtained. The liver was obviously rotated downward. The left lobe lay beneath the dome of the diaphragm and the right lobe filled the right half of the abdomen. The thorotrast also clearly revealed a sharply defined filling defect, measuring approximately 8 cm. in diameter. This lay in the right lobe. A diagnosis of gummatous syphilis of the liver was made and specific therapy was instituted. Following adequate intramuscular and intravenous antiluetic treatment, the patient became entirely free of symptoms. Subsequent roentgenograms taken over a 6-year period clearly demonstrated a progressive diminution in size of the liver and ultimate disappearance of the gumma. Ten years after the injection of thorotrast, the patient, then 68 years of age, was described as being in good health and very active physically and mentally. It is of interest that her blood pressure was subnormal for her age, averaging about 110/60 mm. Hg. Two years later she was admitted to the hospital as an emergency case. She had suddenly experienced weakness and pallor. On admission she was restless and her pulse was rapid. Laboratory studies revealed a hemoglobin of only 37 per cent and a red blood cell count of 1,760,000. Her condition

was very poor. Blood pressure recordings were unobtainable. Treatment with transfusions was of no avail. She died a few hours after entrance with the clinical diagnosis of "shock, the result of internal hemorrhage."

AUTOPSY FINDINGS

At autopsy the body was found to be moderately well nourished and well preserved for 70 years of age. The skin and underlying tissues were pale and bloodless. The peritoneal cavity contained 2000 cc. of fluid and freshly coagulating blood. The *liver* was rotated and displaced to the right, and lay in an almost vertical plane. It was smaller than is normal and weighed only 1050 gm. It was composed of small and large lobes separated by deep and shallow furrows. It presented the picture of hepar lobatum. It was firm, reddish brown, and the surfaces of the individual lobes were smooth. In addition to this displacement and deformity there were three other unusual findings. First, the entire capsular surface showed a fine yellowish white, thread-like marking. Secondly, the right lobe was spotted with brilliant patches of red varying from 0.1 to 2 cm. in diameter. Thirdly, there was a ragged, friable, soft, spongy mass, 5 cm. in diameter, saturated with blood, that merged with the inferior surface of the liver. This was the source of the fatal hemorrhage and this was a primary tumor. Sections of the liver revealed that this soft blood-soaked mass of neoplastic tissue was embedded in its substance, that the many small hemorrhagic areas visible at the surface were equally numerous throughout the entire right lobe of the liver (these proved to be metastases), and that the yellowish white thready lines seen through the capsule traversed the whole liver. Adjacent to the hemorrhagic tumor these lines became broader and more numerous and converged on a solitary, firm, creamy yellow, gritty mass, 2 cm. in diameter. The *spleen*, which weighed 100 gm., was soft, reddish brown, and almost bloodless. Like the liver it showed, both on the surface and on section, innumerable delicate, yellowish white, interlacing thread-like lines throughout the parenchyma. *Lymph nodes* from the hilus of the liver, mesentery, and para-aortal area contained a yellowish white substance. The *bones* were fragile and could be cut easily with a knife. The *marrow* of the ribs, sternum, and vertebrae was red. The *heart* was small and moderately firm. The *lungs* showed one interesting finding grossly. There were several small, solitary, discrete hemorrhages in the right lower lobe which proved to be metastases.

Microscopical Examination

Liver. Before enumerating or describing the very extensive and unusual pathological changes within the liver, it is important to point out that a considerable portion was still well preserved. Three significant

histological lesions were demonstrable: (1) A primary malignant tumor with metastases, (2) a very heavy deposition of thorotrast with severe damage to the liver, and (3) scars of healed syphilitic hepatitis with gumma formation.

The most important of these three findings was the tumor with its regional metastases throughout the liver. There were several noteworthy features of this tumor (Fig. 1). It had originated in an area immediately bordering the largest concentration of thorotrast in the body. It was composed of a single type of cell resembling most closely the Kupffer cell. The cells varied considerably in size; the average was about as large as a liver cell. They varied in shape. Some were nearly round, others were elongated and narrow, still others were very irregular and showed grotesque vacuolated forms with large and small, clear, blistering projections of their cytoplasm. The average cell had very little cytoplasm and this stained mildly basophilic. The texture of the cytoplasm varied from ground glass to finely granular. The outer limits of the cells were poorly defined; often no cell membrane was demonstrable. The nucleus occupied most of the cell and its shape could be compared to that of a potato. The nuclear membrane was sharp, thin, and wire-like. The nucleus stained very lightly except for two or three coarse clumps of chromatin that were suspended in a very delicate web of reticulum. Mitotic figures were rarely seen and these were atypical. Some tumor cells showed degeneration, necrosis, disintegration, and lysis. Karyorrhexis with nuclear fragmentation was a conspicuous feature of this regressive change. Other tumor cells contained inclusions consisting of thorotrast, red blood cells, leukocytes, and hemosiderin. Tumor cells containing thorotrast seemed particularly susceptible to necrosis. While tumor cells appeared singly, they tended to arrange themselves in three common patterns, namely: (a) in clusters so close together that individual cell outlines could not be identified, (b) as walls of irregular blood sinuses, bearing the same relation to liver cells as Kupffer cells elsewhere, and (c) as walls of blood vessels replacing normal endothelium. Everywhere they showed a tendency to cling to pre-formed surfaces, whether cells, basement membranes, or reticulum. They invaded, destroyed by lysis, and crudely replaced normal sinus endothelium (Fig. 2). They invaded the trabeculae of liver cells, broke up lobules, and destroyed normal patterns (Fig. 3). Their most conspicuous characteristic was to break normal endothelial barriers and produce hemorrhages which showed no sign of coagulation (Fig. 4). They grew within the lumina of branches of the portal vein and metastasized freely throughout the liver (Fig. 5). The effect of the tumor in the liver was fourfold (Fig. 6). First, its very presence destroyed

liver tissue; secondly, the hemorrhages led to compression and atrophy of liver cells; thirdly, by interfering with local circulation there was infarction and necrosis; and lastly, by destruction and rupture of the capsule of the liver there was a fatal intra-abdominal hemorrhage.

The second significant finding was the extensive deposition of thorotrast. No portion of the liver was entirely free of it, but in some areas there was so little that the normal structure was well preserved. In other fields there was so much of this material that all structural detail was entirely lost and in such areas no living or necrotic cells were recognizable (Fig.7). The thorotrast appeared as tiny granules and globules closely packed together. It was rather colorless but at times acquired a faint eosinophilic tint. As the index of refraction was quite different from that of the tissues, it could be visualized very clearly in the tissues by controlling the amount of light. Much of this substance lay quite free in the tissues; the rest was taken up by histiocytes, Kupffer cells, and tumor cells. Some cells contained little, others were so distended with it that only the cell outline was recognizable. In fields where the cells were packed, it was impossible to distinguish one cell from another. Cells distended with this material died and their contents spilled into the surrounding space. The greatest concentration was in areas of old inflammatory scar tissue. Next in degree of concentration was the region about the central vein where it replaced the liver cells of the central zone, and at times of the midzones of the lobule (Fig. 8). In this area there was some condensation and hyalinization of the stroma, but there was no significant increase in fibrous tissue. A third site was in the capsule and portal connective tissue, but though the amounts here varied considerably, they were not to be compared with the larger quantities in the scars and central zones (Fig. 9). In many of the portal areas thorotrast was deposited in histiocytes or was free. In either case it lay embedded in an excess of dense, homogeneous, hyaline connective tissue. This increase in portal connective tissue was associated with an obliteration of lymphatics and a narrowing or occlusion of veins. Occasionally in such areas a vein was partially or completely occluded by a thrombus. The increase in collagen in association with thorotrast was much greater in the capsule and portal areas than in the central zones. Liver cells bordering collections of thorotrast showed several forms of degeneration, disintegration, and necrosis. One of these was characterized by the accumulation of lipids and hyalin in the cells (Fig. 10).

The third important finding in the liver was the presence of large and small, old healed inflammatory scars. These scars accounted for the gross nodular deformity. Some scars contained little thorotrast,

others were saturated with it. There was nothing in any of the sections to suggest an active syphilitic infection.

Spleen. There was a heavy deposition of thorotrast in fibrous tissue outside the splenic capsule, in the capsule, in and about the trabeculae, in the pulp, and in the follicles, but the greatest concentration was centered about the small vessels (Fig. 11). No part of the spleen was spared. As in the liver, thorotrast tended to collect in tracts and clusters. Sometimes it was confined to histiocytes, but often, and especially in fibrous tissue, it lay quite free. The detailed structure of the pulp was poorly defined, but almost everywhere there was a relative increase in supporting collagen. This was especially true of capsule and trabeculae. The follicles were small and relatively numerous. Each showed a small central core of primitive cells bordered by a loose ring of lymphocytes. Some of the sinuses contained developing blood cells, and in one or two areas in the pulp there were found clusters of primitive erythroblasts. There was a moderate deposition of iron pigment, and at times histiocytes contained both thorotrast and hemosiderin. An interesting observation was the complete absence of polymorphonuclear leukocytes in all sections.

Lymph Nodes. Thorotrast was deposited all through the nodes, but there was the same tendency here as in the spleen and liver for it to be concentrated in masses (Fig. 12). Thorotrast was found in circulating monocytes within the peripheral and traversing sinuses. Almost as conspicuous as the thorotrast was the diminution in the total number of lymphocytes (Fig. 13). The lymphoid tissue was confined to small scattered nodules embedded in a web of reticulum. There was some bleeding into the node and, although there was a moderate reticulum and endothelial cell hyperplasia, there was no suggestion of connective tissue proliferation or fibrosis.

Bone Marrow. Solitary histiocytes and large and small nests of histiocytes laden with thorotrast were scattered throughout the marrow (Fig. 14). There was a very uneven distribution and a very definite diminution in hematopoietic tissue. There was too much fat in the marrow, even for this age. The capillaries and sinuses were numerous and dilated, and capillary bleeding was common. Erythropoiesis was abnormal. There were small nests of erythroblasts and megaloblasts free of hemoglobin. There also were clusters of normoblasts, but many of these showed little tendency to exhibit normal maturation and some appeared to be undergoing disintegration. In many fields there were no myeloblasts, no myelocytes, and no cells of the myelocytic series. There were very few mature polymorphonuclear leukocytes, and eosinophils were entirely lacking. The number of megakaryocytes was

diminished, and of those present the majority were quite immature. There were loosely arranged nests of lymphocytes replacing normal marrow (Fig. 15). The over-all picture was one of too few cells, and of these, many were very primitive, many lacked evidence of normal maturation, and many, especially of the red cell series, showed premature death. There was no hemosiderosis and no erythrophagocytosis.

Bones. There was generalized osteoporosis and in many areas trabeculae had entirely disappeared (Fig. 17). The trabeculae were thin and delicate, and some showed patches from which all bone cells had disappeared. Their margins were ragged and rough, and the ground substance showed a linear marking with a tendency to split longitudinally and even to break up. Histiocytes laden with thorotrast often lay adjacent to bone, sometimes along the surface of trabeculae, at other times about capillaries along the haversian canals. It should be pointed out that no thorotrast was found within the ground substance of bone.

Adrenal Glands. There was little thorotrast in the adrenal medulla and in the endothelial cells of the sinuses throughout the cortex. Islands of medullary tissue were replaced by nests of lymphocytes and plasma cells, and by histiocytes laden with thorotrast (Fig. 16). There was degeneration, atrophy, and fibrosis of portions of the zona glomerulosa containing thorotrast (Fig. 18).

Kidney. The normal structural detail of the kidney was well preseved, but there were two changes worthy of description. In blood vessels (Fig. 19) there was a disappearance of stained cytoplasm from the smooth muscle fibers of the walls; the elastica was interrupted and and in places absent, and the lumina were abnormally wide. The second change was that an occasional glomerulus showed a deposition of thorotrast associated with focal collapse and hyalinization of the glomerular tuft.

Lungs. In sections of the lungs taken through areas of hemorrhage there were tumor cells lining alveolar spaces distended with blood. There was a disappearance of smooth muscle from the walls of blood vessels (Fig. 20) and bronchi, and a replacement with hyaline tissue. No thorotrast was found in sections taken from the periphery of the lung.

Heart. Beneath the endocardium the muscle fibers and their cross striations were well preserved but elsewhere the fibers were swollen and their cross striations were either lost completely or were cloudy and barely discernible. No thorotrast was found, but there was a light collection of lymphocytes and plasma cells about some of the small vessels in the interstitial tissue. Small arteries were dilated and their walls were hyalinized and devoid of smooth muscle.

Final Diagnoses

Fatal intra-abdominal hemorrhage, from an endothelial-cell sarcoma of the liver showing multiple regional metastases. Thorotrast deposition and irradiation, with degeneration of liver, spleen, lymph nodes, bone marrow, bones, adrenals, kidney, and blood vessel walls. Old healed syphilitic hepatitis (hepar lobatum).

DISCUSSION

All four significant lesions in this patient—the fatal hemorrhage, the malignant tumor, the thorotrast deposition, and syphilis—were centered in the liver. The hemorrhage was very large and sudden, and the tumor was its obvious source. This was filled with blood even after its outer surface, which was formed by the liver capsule, had ruptured into the peritoneum.

A hemorrhagic sarcoma apparently having origin in sinus endothelium is a very rare primary tumor of the liver. In this tumor the cells resembled Kupffer cells in both structure and function. They had the property of phagocytosis and could build crudely constructed sinuses. They tended to retain the same intimate association with liver cells as is shown by normal sinus endothelium. Their most striking characteristic was to destroy normal endothelium and produce multiple hemorrhages.

The significance of the thorotrast in the liver must not be underestimated. Most important was the fact that the tumor had originated in immediate association with the largest single deposition of this material in the body. Secondly, thorotrast had severely damaged the liver cells by slowly progressive necrobiosis which at the time of death was still active. The almost negligible regeneration of liver cells may also be attributed to its presence. Thorotrast had led to fibrosis of portal areas with narrowing and occlusion of veins and lymphatics and subsequent interference with the portal circulation.

In respect to the original syphilitic lesion, all that remained were coarse scarring and contractures leading to the well known hepar lobatum. That the liver was much below normal in size and weight can be explained only by the scarring and by progressive destruction of liver cells without significant regeneration.

Thorotrast in the reticulo-endothelial system of bone marrow, spleen, liver, and lymph nodes had seriously affected hematopoiesis. Much of this material was confined to the fixed cells, much of it was free, but some of it was found in circulating histiocytes, clearly indicating that thorotrast was in a continual process of migration.

Grossly, the bones were fragile and could be cut or broken easily;

histologically, there was direct evidence of necrosis where thorotrast and bone lay side by side.

In addition to these changes which were associated with thorotrast in immediate contact with tissues, there was the possible remote effect that thorotrast might have had on the cardiovascular system. Histologically, three changes were repeatedly observed in vessels in different parts of the body. First, there was degeneration and disappearance of smooth muscle fibers from the walls of both veins and arteries. Secondly, there was degeneration and interruption of the elastica in the walls of smaller arteries. Thirdly, there was a tendency on the part of arterioles and capillaries to dilate and bleed. It is of interest to correlate these changes with the clinical observation that in later years the patient's blood pressure had been consistently low.

Thorotrast as an Injurious Substance

There can be little doubt from a study of this case that thorotrast can do harm. The observations of Jacobson and Rosenbaum [5] can attest to that, for they found fibrotic changes in liver, spleen, lymph nodes, and arteries in a patient who 5 years before had received 75 cc. of this substance. Thorotrast, or thorium dioxide, is distinctly radioactive. In this respect it closely resembles uranium and radium, the activity of which depends on the continual liberation of ionizing radiations known as alpha, beta, and gamma emanations. In the body it is the gamma ray which is known to cause most harm. The amount of thorotrast injected into the body for diagnostic purposes varies, but the maximum doses employed are known to have a gamma ray equivalent to about 1 to 3 μg. of radium. The general effects of irradiation injury in the body are well known and parallel very closely the findings in our patient. For example: Bone marrow shows hypoplasia, dysplasia, and a disappearance of eosinophils; capillaries dilate and show a tendency to bleed; lymphoid tissue shrinks through injury to lymphocytes; bones become atrophic and break; glandular tissue degenerates; and smooth muscle may disappear.

Because of the property of spontaneous disintegration possessed by radioactive substances, it was of interest to know whether thorotrast, after a period of 12 years in the body, was still radioactive. To answer this question, blocks of liver tissue that had been preserved in formalin as long as 12 months after the autopsy, were tested with a Geiger-Mueller counter. This is a comparatively simple electrical apparatus, named after its inventors, for measuring and observing cosmic emanations. Each piece of liver gave a positive recording and the highest reading was obtained from the area of greatest thorotrast concentration. This was also the site of the primary malignant tumor.

Thorotrast as a Sarcogenic Agent

In 1929 Martland and Humphries [6] were the first to report the appearance of osteogenic sarcoma, attributed to radium and mesothorium, in 2 of 15 girls employed at painting watch dials with luminous radioactive paint. This report was followed 3 years later by a paper by Sabin, Doan, and Forkner [7] who described the appearance of osteogenic sarcoma in 2 of 7 rabbits, surviving 11 and 19 months respectively, that had been injected intravenously with radium chloride and mesothorium. Ross,[8] in 1936, implanted a platinum tube containing 0.1 mg. of radium under the periosteum of the rib of a rabbit, and 2 years later the tube was found encased in a large, actively growing osteogenic sarcoma. More recently, Dunlap, Aub, Evans, and Harris [9] produced osteogenic sarcoma in 9 of 13 male Wistar rats after feeding each animal 100 μg. of radium. There is no longer any question about the appearance of malignant tumors in man and animals subsequent to the injection or ingestion of radioactive substances. It is apparent, however, that in each of these cases the primary tumor has been in bone. This might suggest tissue specificity, but there is another and much more probable explanation, for Martland [10] has shown that when radium salts are ingested orally, the distribution of radium in the body is like that of lead. After absorption, it is phagocytized by cells of the reticulo-endothelial system and then it enters the skeletal system where it is concentrated and, in part at least, may be permanently retained. This is important in skeletal tumors induced by radium, for it is in bone and from bone that the osteogenic sarcomas arise.

The significance of such factors as concentration and localization of this sarcogenic agent in respect to the site and type of tumor produced has been demonstrated again by Roussy, Oberling, and Guérin,[11] who successfully produced peritoneal and subcutaneous sarcomas in 8 of 15 surviving mice that had been given intraperitoneal and subcutaneous injections of thorium dioxide. In our patient the greatest concentration of thorotrast was in the liver and it was in this organ that the primary malignant Kupffer cell sarcoma arose.

Thorotrast as a Diagnostic Aid

The most widespread use of thorotrast has been in the field of gastroenterology in the detection of diseases of the liver. In urology it seemed at first to be an ideal substance for pyelography, but soon its use was discarded because it tended to cause exacerbations of existing infections, to damage collecting tubules, to lead to obstruction, and to obscure lesions within the kidney. In certain diseases of the central nervous system it has been used in both diagnosis and localization. Here it has been injected locally, into vessels and even into the ven-

tricles; but for cerebral angiography, encephalography, and ventriculography its use has been generally condemned. It is still used, however, with great advantage, in very small quantities, to outline a cyst or abscess of the brain. Its most recent use has been in a study by Davis and Potter [12] of intra-uterine respiration and gastrointestinal activity. After injecting thorotrast into the amniotic sac it soon appears in both lungs and intestines of the fetus.

Summary and Conclusion

A case is reported of a patient who had been given thorotrast for the visualization of the liver. With the aid of this diagnostic procedure, combined with positive serological tests, it was possible to make an accurate diagnosis of hepatic syphilis with gumma. Following specific therapy, the patient made a clinical recovery and for 12 years lived a reasonably normal life. At the age of 70, death came suddenly. Autopsy findings confirmed the diagnosis of syphilis and in addition revealed a primary hemorrhagic endothelial-cell sarcoma of the liver, the source of fatal hemorrhage, and very widespread irradiation injury affecting particularly the liver and hematopoietic system. Evidence is produced from a study of this case to support the debatable contention that thorotrast, in sufficient quantities, as a radioactive substance, is injurious. Evidence is also produced to show that thorotrast, like other radioactive substances, in sufficient time may act as a sarcogenic agent.

REFERENCES

1. Blühbaum, T., Frik, K., and Kalkbrenner, H. Eine neue Anwendungsart der Kolloide in der Röntgendiagnostik. *Fortschr. a. d. Geb. d. Röntgenstrahlen,* 1928, 37, 18–29.

2. Reeves, D. L., and Stuck, R. M. Clinical and experimental results with thorotrast. *Medicine,* 1938, 17, 37–73.

3. Yater, W. M. Fundamentals of Internal Medicine. D. Appleton-Century Co., New York, 1944, ed. 2, p. 402.

4. McClure, C. W., Jankelson, I. R., and Osgood, H. Diagnostic advantages of thorotrast. *Rev. Gastroenterol.,* 1944, 11, 17–22.

5. Jacobson, L. E., and Rosenbaum, D. Post-mortem findings and radioactivity determinations five years after the injection of thorotrast. *Radiology,* 1938, 31, 601–607.

6. Martland, H. S., and Humphries, R. E. Osteogenic sarcoma in dial painters using luminous paint. *Arch. Path.,* 1929, 7, 406–417.

7. Sabin, F. R., Doan, C. A., and Forkner, C. E. The production of osteogenic sarcomata and the effects on lymph nodes and bone marrow of intravenous injections of radium chloride and mesothorium in rabbits. *J. Exper. Med.,* 1932, 56, 267–289.

8. Ross, J. M. The carcinogenic action of radium in the rabbit: The effect of prolonged irradiation with screened radium. *J. Path. & Bact.,* 1936, 43, 267–276.

9. Dunlap, C. E., Aub, J. E., Evans, R. D., and Harris, R. S. Transplantable osteogenic sarcomas induced in rats by feeding radium. *Am. J. Path.*, 1944, 20, 1–21.

10. Martland, H. S. Radium Poisoning. In: Blumer, G. (ed.) The Practitioners Library of Medicine and·Surgery. Supplement—Index. D. Appleton-Century Co., New York, 1938, pp. 208–219.

11. Roussy, G., Oberling, C., and Guérin, M. Action cancérigène du dioxide de thorium chez le rat blanc. *Bull. Acad. de. méd., Paris,* 1934, 112, 809–816.

12. Davis, M. E., and Potter, E. L. Intrauterine respiration of the human fetus. *J. A. M. A.,* 1946, 131, 1194–1201.

[Illustrations follow]

DESCRIPTION OF PLATES

All photomicrographs were made from sections stained with eosin and methylene blue.

PLATE 95

FIG. 1. Liver, showing nests of tumor cells bathed in blood. In the lower left-hand corner there are three groups of liver cells surrounded by tumor cells and blood. The tumor cells tend to group in clusters and to cover and surround liver cells with which they come in contact. The tumor cell is often as large as a liver cell; it has little basophilic cytoplasm, that varies from a ground-glass appearance to very finely granular. Its cell membrane is indistinct and when the cells are clustered it is impossible to see the lines separating one from another. *The nucleus is large and occupies most of the cell. It is lightly* stained. The nuclear membrane is sharp and wire-like. In shape, the nuclei resemble potatoes. There is little chromatin and this forms coarse clumps suspended in delicate interlacing threads. Mitotic figures are very seldom seen and then they are asymmetrical and hyperchromatic. $\times$ 275.

FIG. 2. Liver. In the lower right-hand corner there are trabeculae of liver cells showing compression atrophy. The remainder of the field is made up of loose tumor cells bathed on all sides by noncoagulated ·blood, and of a few small clumps of degenerating liver cells—all that remains of the lobule. $\times$ 140.
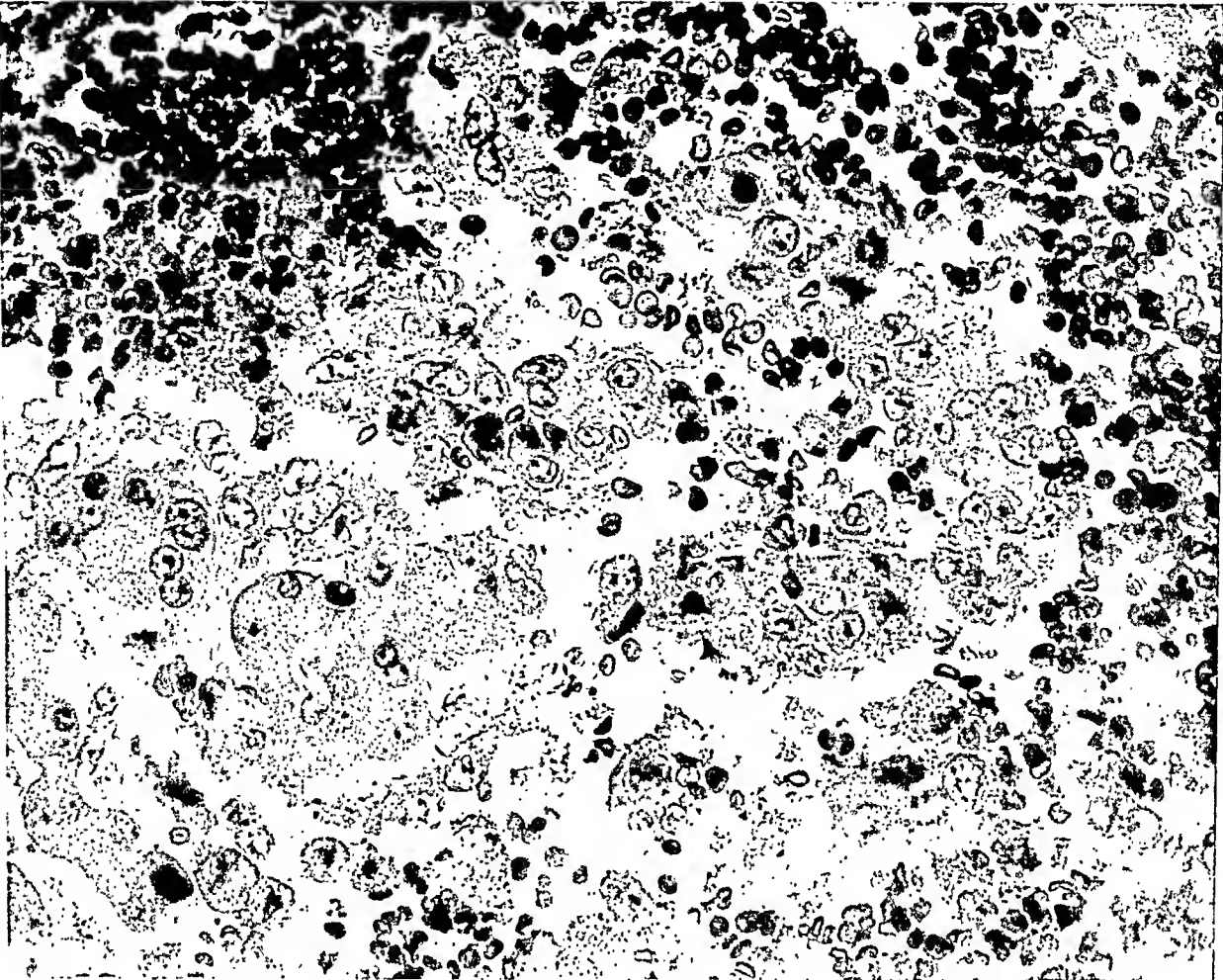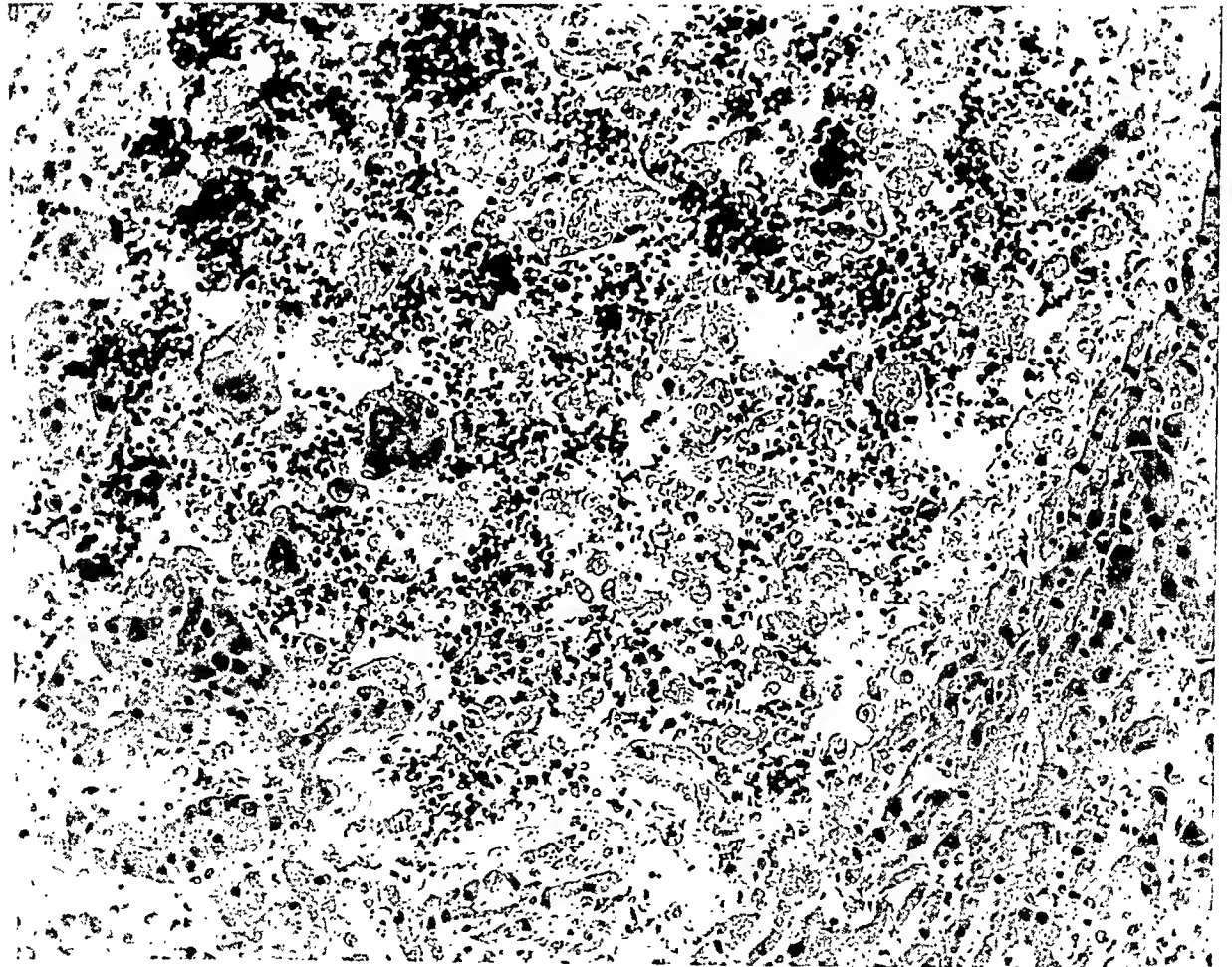
PLATE 96

FIG. 3. Liver, showing an area in which the normal trabecular pattern is broken up into small cords or islands of liver cells separated by spaces filled with blood and bordered in part by tumor cells. An occasional film of fibrin coats an island of degenerating liver cells. Small deposits of thorotrast are visible in the surrounding tissue. × 90.

FIG. 4. Liver, showing one of many hemorrhages. This hemorrhage lies very close to a portal area, below. The surrounding liver cells show compression. In the area of hemorrhage there are a number of tumor cells almost completely hidden by blood. A few necrotic and disintegrating liver cells are seen adjacent to the margin of the hemorrhage. There are small deposits of thorotrast, both to the right and left of the hemorrhage. × 140.

3

4

MacMahon, Murphy, and Bates

Sarcoma of Liver Following Thorotrast
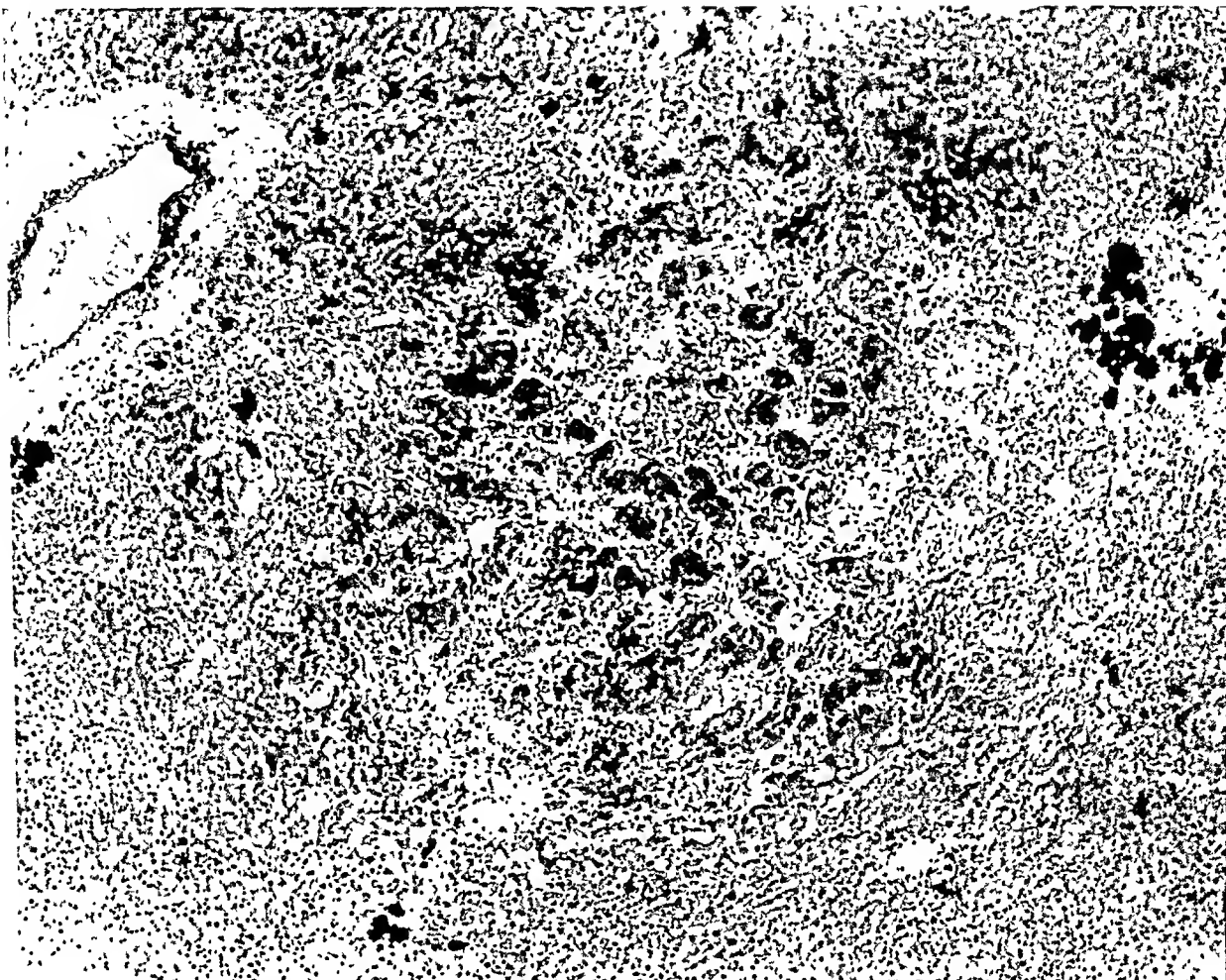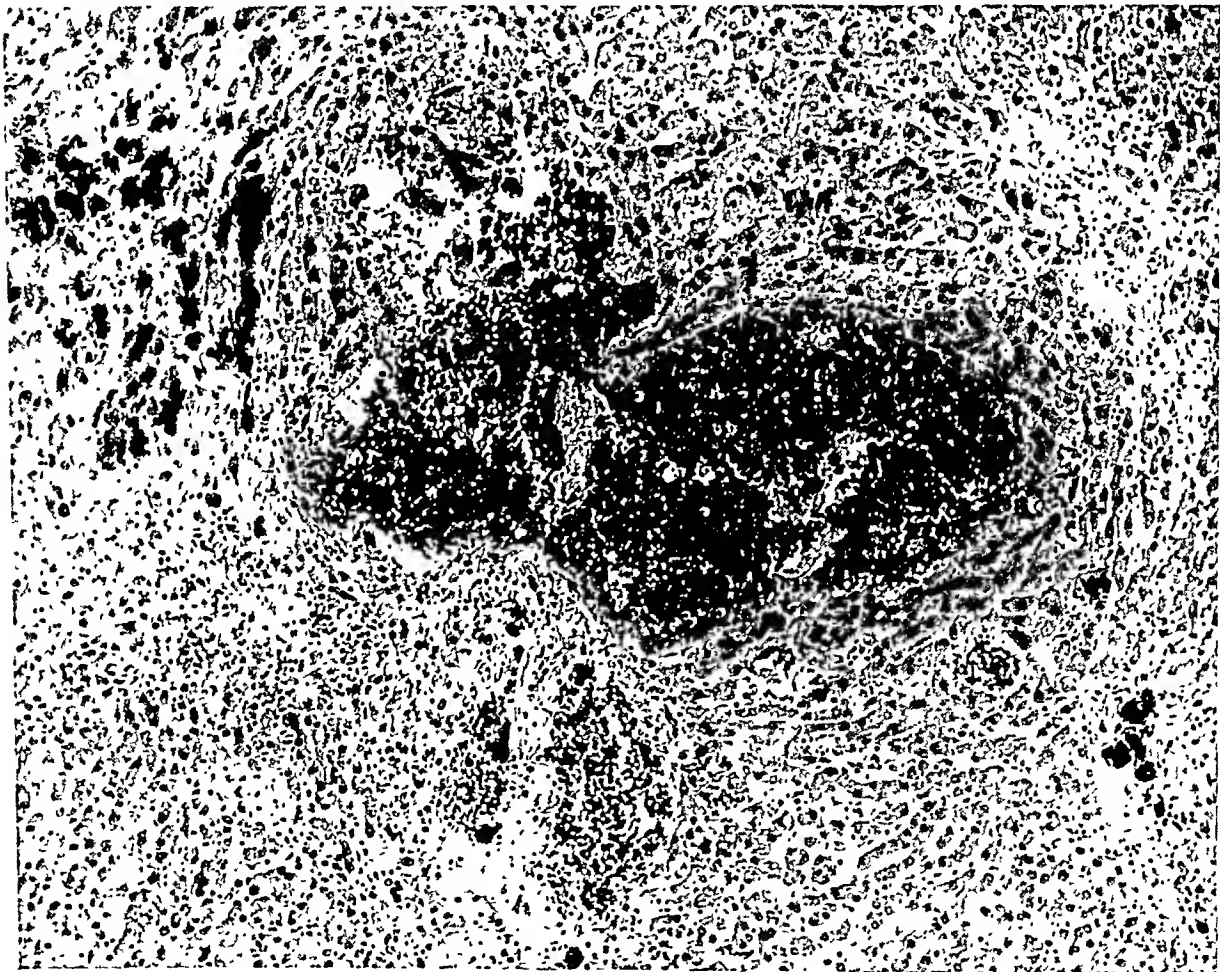
PLATE 97

FIG. 5. Liver, showing a portal area with some adjacent liver cells. A branch of the portal vein crosses and divides at about the center of the field. The lumen of the vein is dilated and almost obstructed by tumor cells. Some of these cells line the walls of the vessel, others lie free and are surrounded by blood. There is no fibrin deposition or coagulation. × 185.

FIG. 6. Liver at a very low power, showing in a single field several deposits of thorotrast, breaking up of the normal trabecular and lobular pattern of liver cells into small cords and islands, necrosis and lysis of liver cells, massive hemorrhage completely devoid of clotting, and a growth of tumor cells throughout the section. The over-all picture has a very superficial resemblance to placental tissue with its chorionic villi embedded in a sea of blood. × 45.

5
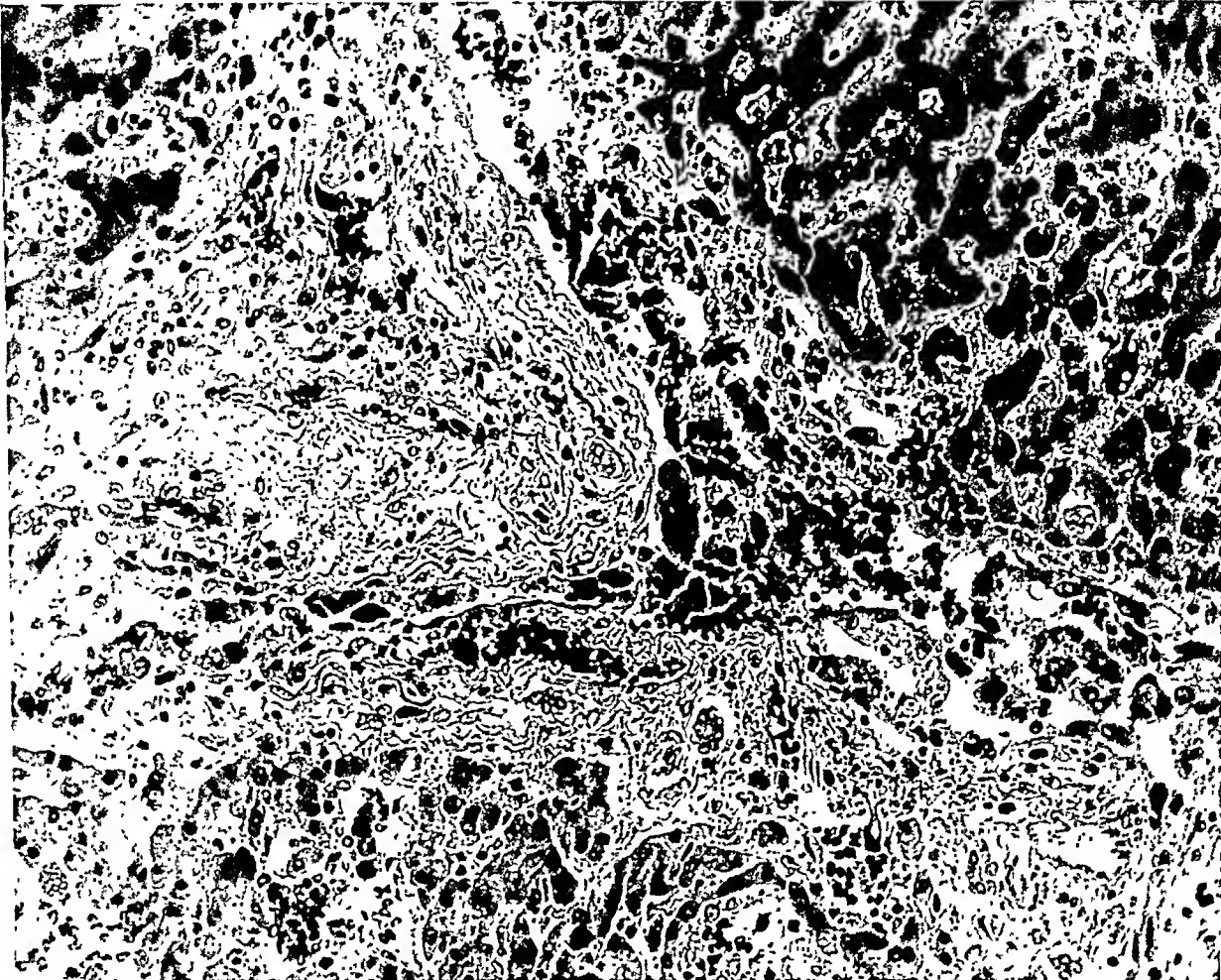
6
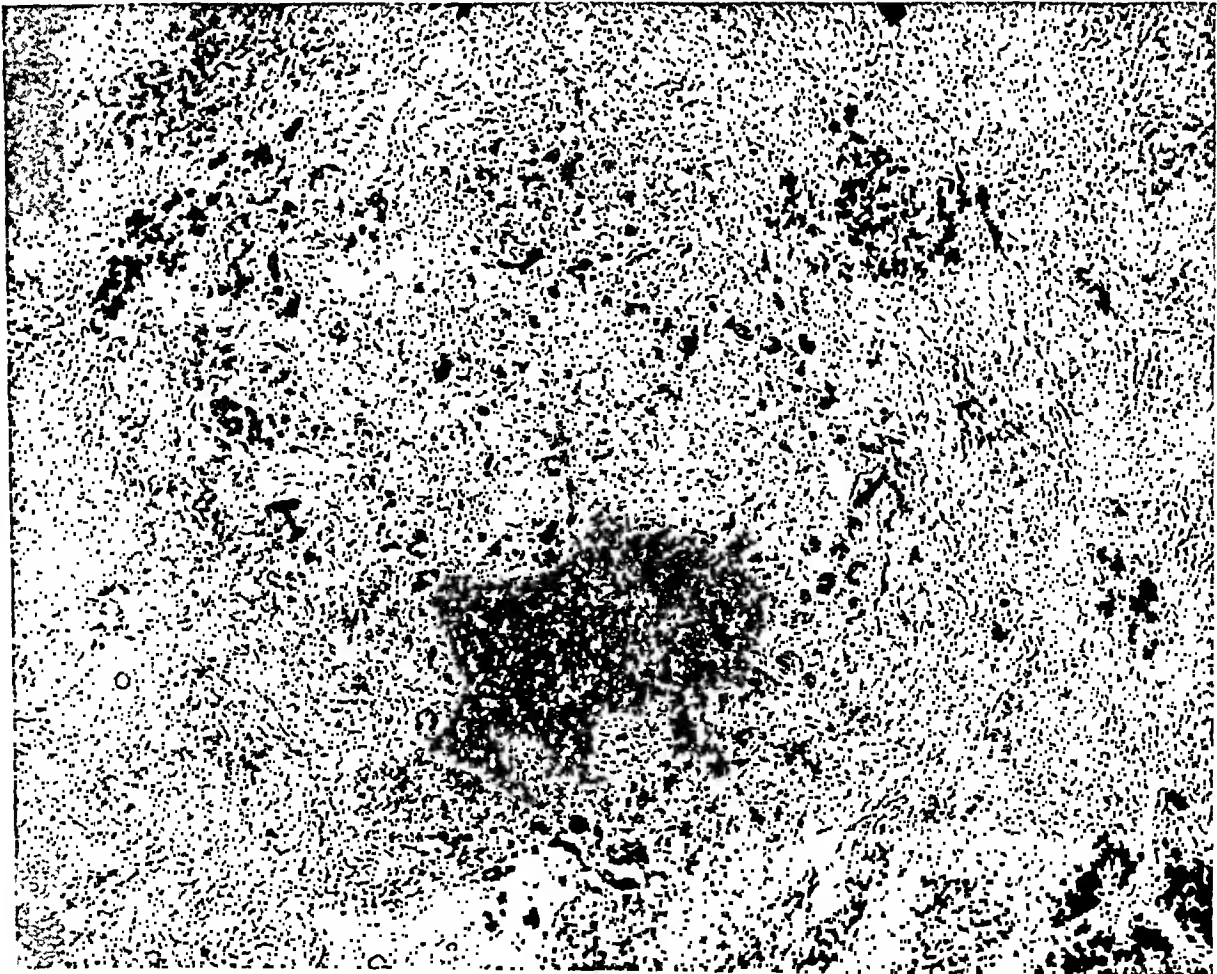
PLATE 98

FIG. 7. An area of the liver showing an unusually heavy deposit of thorotrast. All normal landmarks are lost and not a single hepatic cell is demonstrable. The thorotrast. like slush. lies enmeshed in poorly stained hyaline fibrous tissue. × 275.

FIG. 8. Liver, showing a central vein almost completely surrounded by a deposit of thorotrast and necrotic liver cells. This central zone is bordered by a midzone from which almost all liver cells have disappeared, leaving a reticulum framework infiltered with viable and necrotic histiocytes and plasma cells. Liver cells bordering this area of clearing show necrobiotic changes. There is no evidence of proliferation of liver cells in the outer peripheral zone. There is no increase in fibrous tissue or reticulum. The picture is one of destruction with little secondary resorption. × 140.
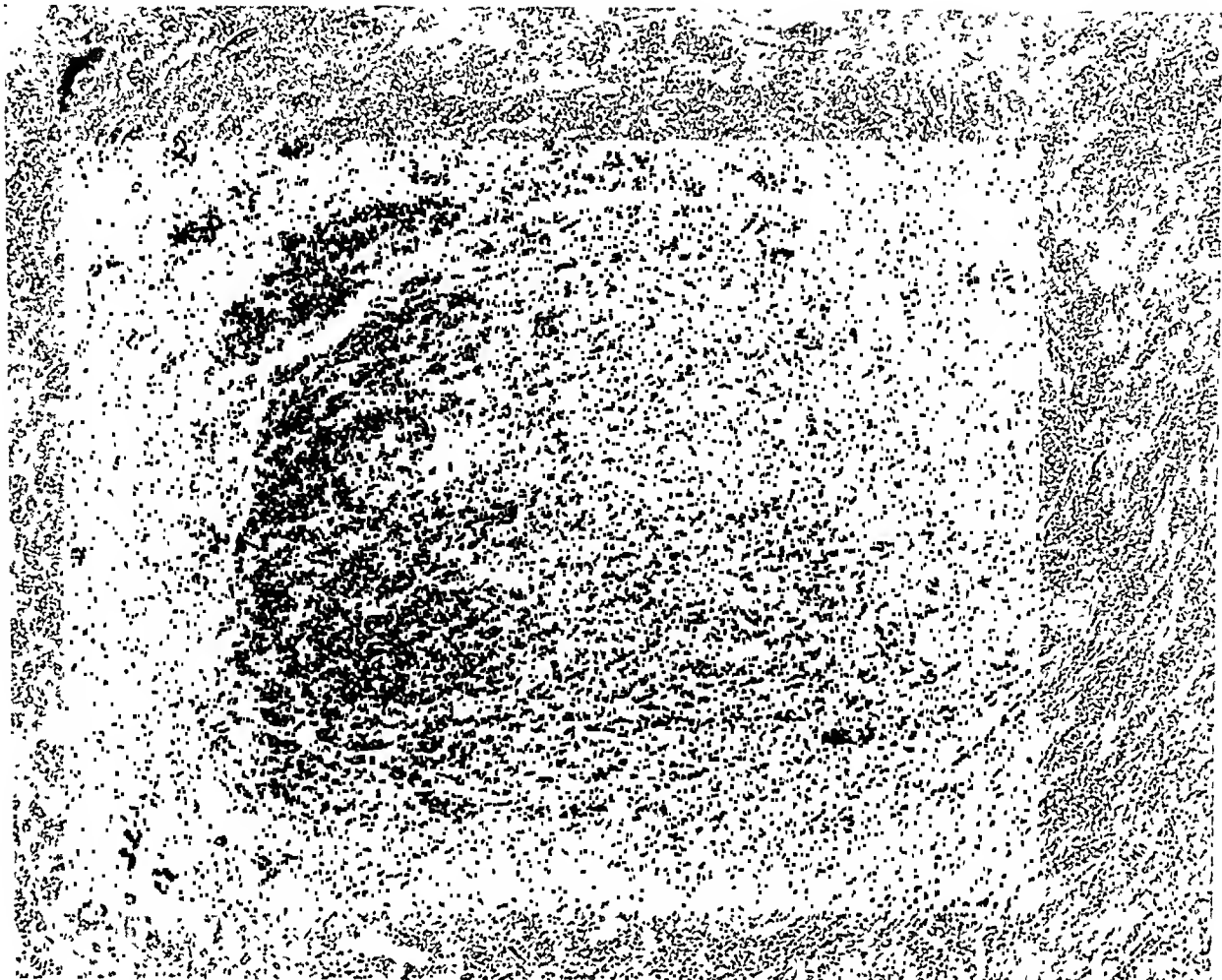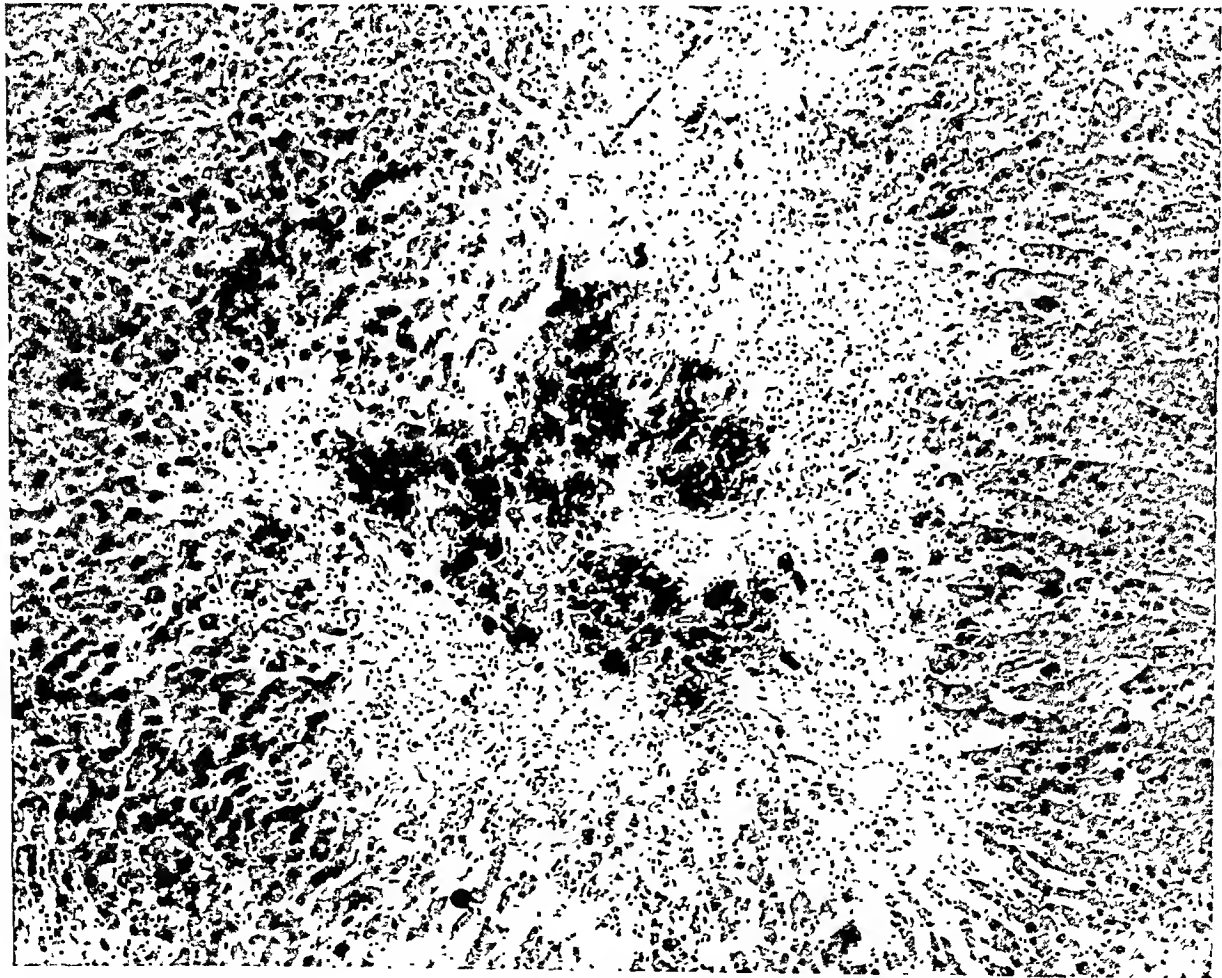
PLATE 99

FIG. 9. Liver, showing a heavy deposit of thorotrast in the central zone of a lobule and smaller deposits in adjacent portal areas. In the large central area both liver cells and endothelial cells have disappeared. Much of the thorotrast lies loosely enmeshed in a condensation of swollen reticulum. The bordering liver cells show regressive changes and necrobiosis. Their nuclei show karyorrhexis, and fragments of chromatin are scattered throughout these cells. × 90.

FIG. 10. Liver, showing a group of liver cells undergoing fatty degeneration, hyaline degeneration, necrosis, and disintegration. There is a sprinkling of thorotrast in this field but no tumor cells are demonstrable. In the upper portion there is a light condensation of stroma as well as a scattering of lymphocytes and plasma cells where liver cells have disappeared. × 370.

FIG. 11. Section of spleen. The central portion of the field is occupied by an accumulation of thorotrast. Most of it lies free; some is confined to the cytoplasm of histiocytes, the nuclei of which it is almost impossible to identify. Red blood cells are freely scattered throughout this mass. In the periphery there are lymphocytes, plasma cells, and, in one corner, a small arteriole showing hyalinization of its wall. × 275.

FIG. 12. Section of lymph node, showing a large deposit of thorotrast. Most of this is in cells—histiocytes. An examination of the individual histiocytes containing this material shows all stages of nuclear degeneration with pyknosis and ultimate lysis. For some time the thorotrast remains within the cytoplasm of the dead cell; then, with rupture of the cell membrane, it spreads into the surrounding area. In this field there are many plasma cells, some of which are giant forms with single and multiple nuclei. Although thorotrast is never found in these cells, they show active proliferation with typical and atypical mitotic figures. In the same area there are degenerating and disintegrating plasma cells, so that the over-all number remains about constant. In this field there is little hemosiderin in histiocytes. An occasional histiocyte contains both hemosiderin and thorotrast. × 90.

9
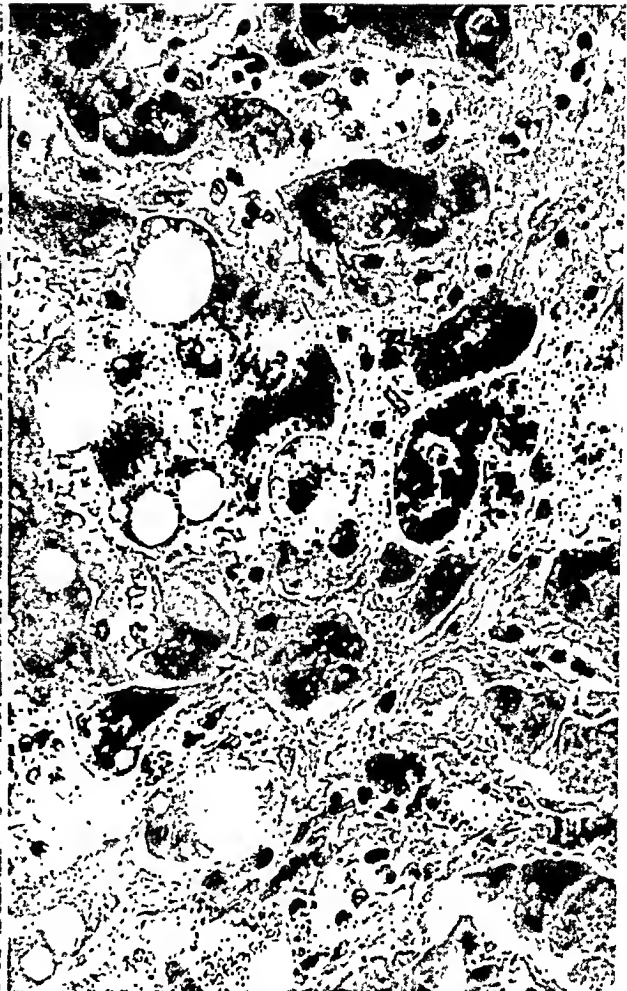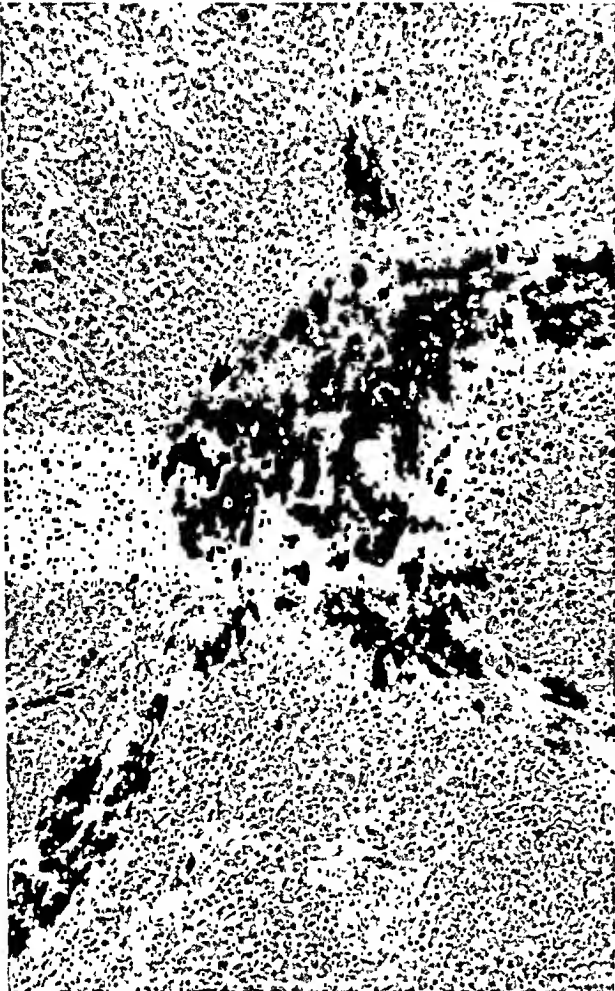
11
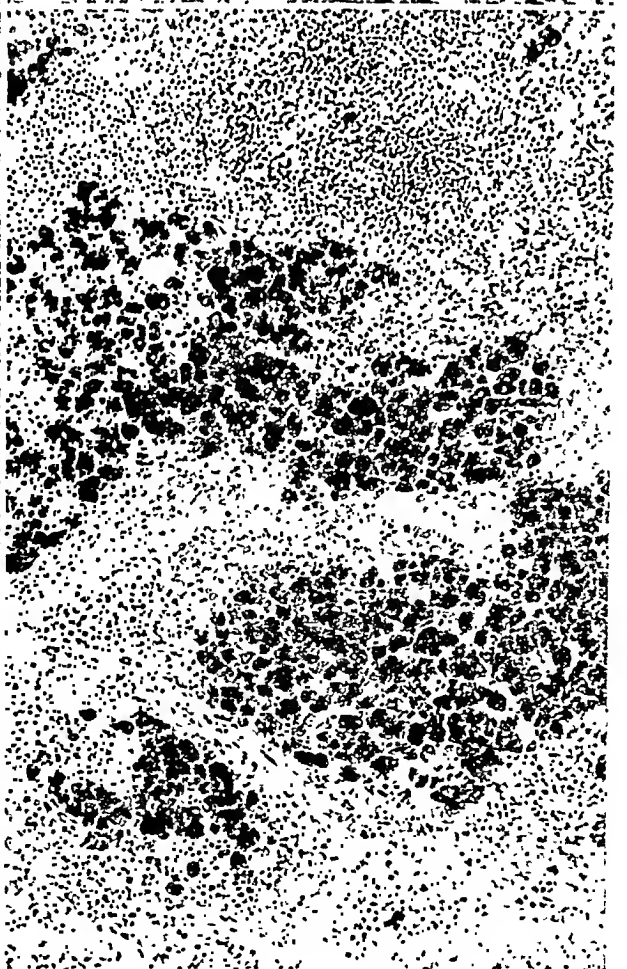
PLATE 100

Fig. 13. Section of lymph node, showing several deposits of thorotrast. Most of this is in cells but some of it is free. Histiocytes containing very small quantities of thorotrast lie free in the sinuses, indicating a continual redistribution of this material. The lymph node shows atrophy of lymphoid tissue with an absolute increase in sinus endothelial cells. Red blood cells are freely scattered throughout the section and some of these are already phagocytosed by histiocytes. Plasma cells are unusually numerous in the capsule. There is no increase in fibrous tissue. × 90.

Fig. 14. Vertebral bone marrow, showing in the center of the field a number of histiocytes singly and in clusters, laden with thorotrast. Roughly 75 per cent of the surrounding cells are erythrocytes; the rest are erythroblasts, normoblasts, megakaryocytes, myelocytes, polymorphonuclear leukocytes, and fat cells. Fluid containing threads of fibrin lies in the intercellular spaces. × 275.

13

14

MacMahon, Murphy, and Bates

Sarcoma of Liver Following Thorotrast
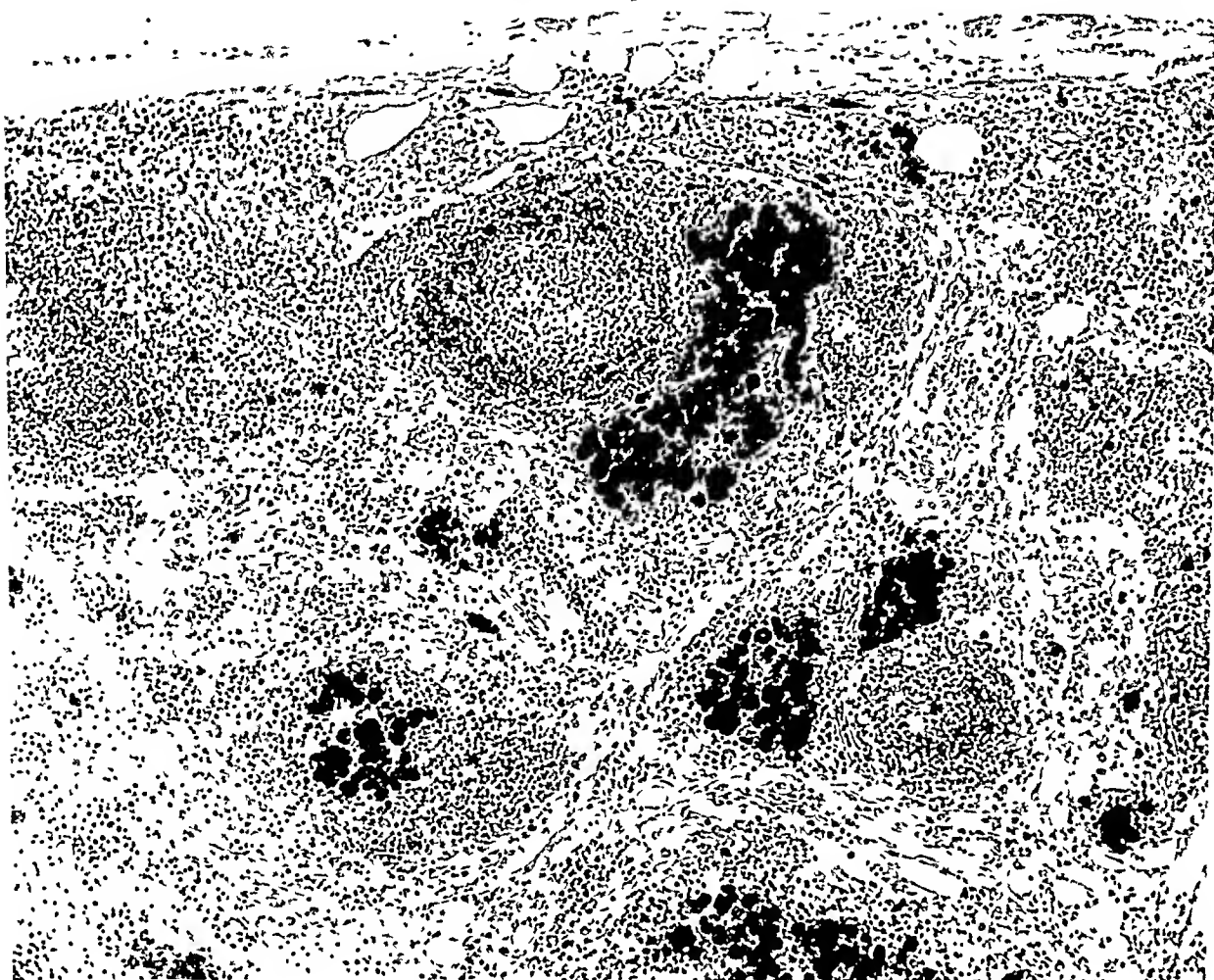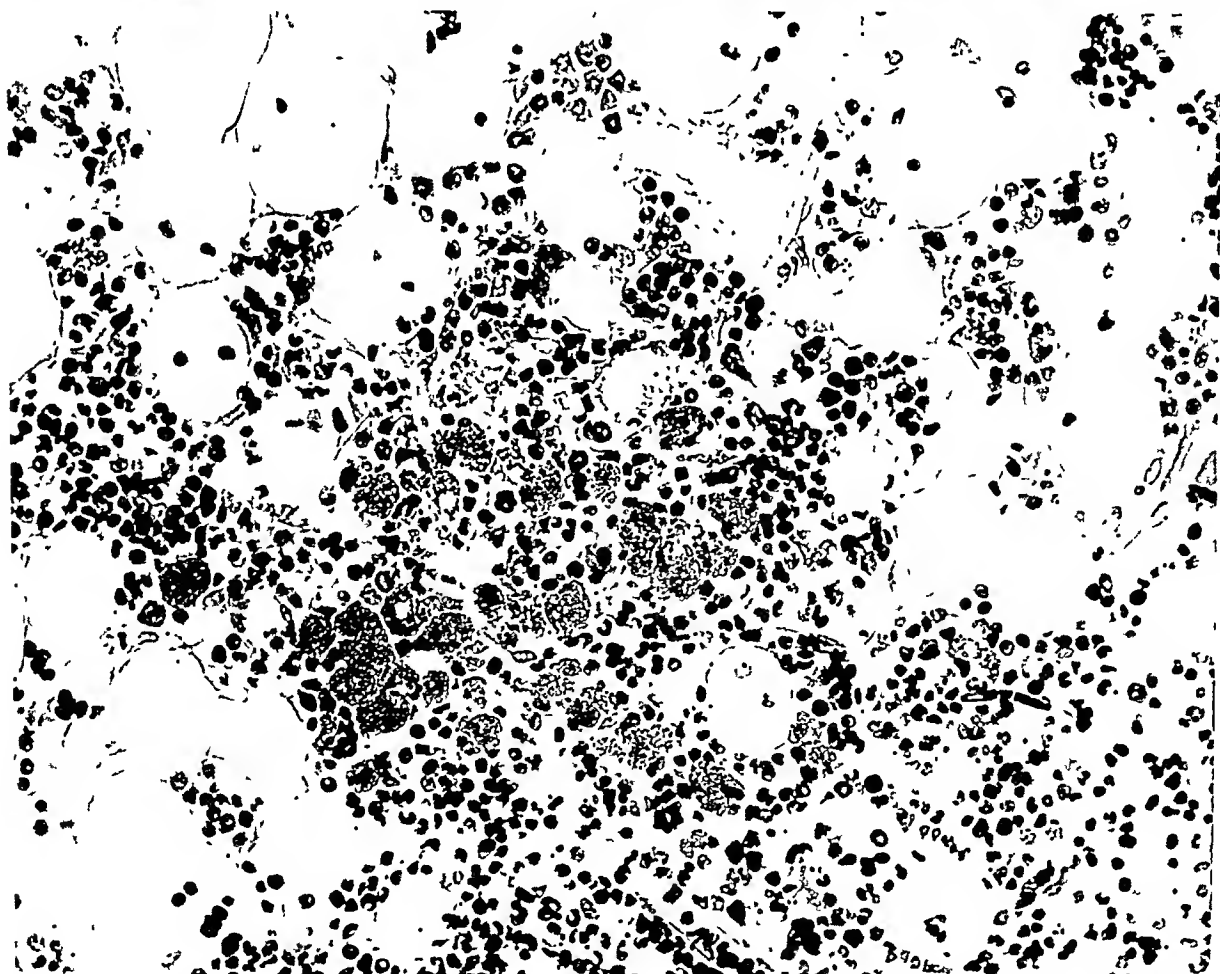
PLATE 101

FIG. 15. Vertebral bone marrow, showing in the center of the field replacement of normal marrow by an island of mature lymphocytes loosely held together by a poorly defined, somewhat hazy, reticulum web. Red blood cells are scattered freely throughout the field. In the lower right-hand corner there is a tiny fragment of bone. $\times$ 275.

FIG. 16. Section of adrenal, showing a field from the medullary area. The pheochromocytes here are almost completely replaced by lymphocytes and small deposits of thorotrast. The wall of the central vein is infiltrated with lymphocytes. $\times$ 275.

15

16

MacMahon, Murphy, and Bates   Sarcoma of Liver Following Thorotrast
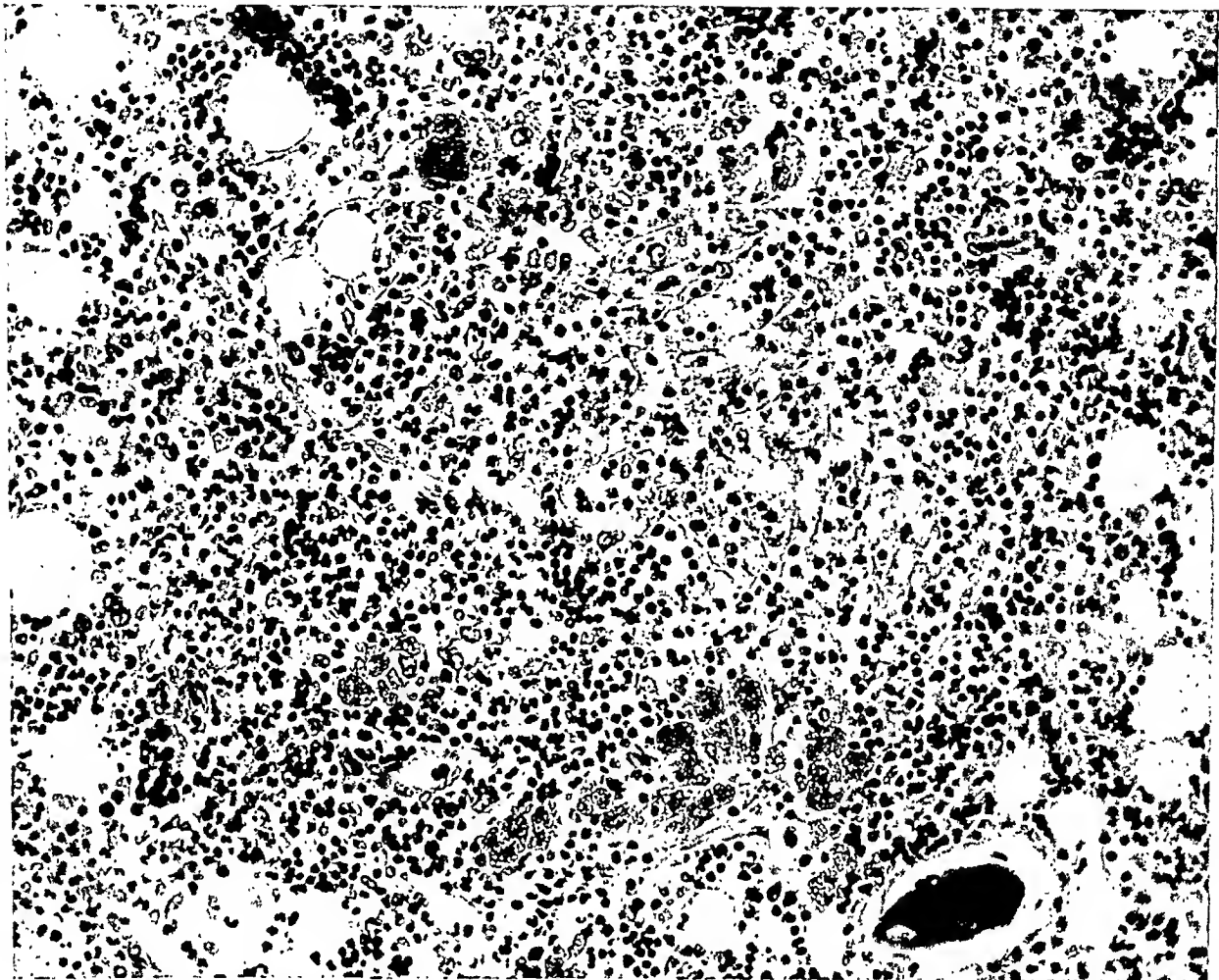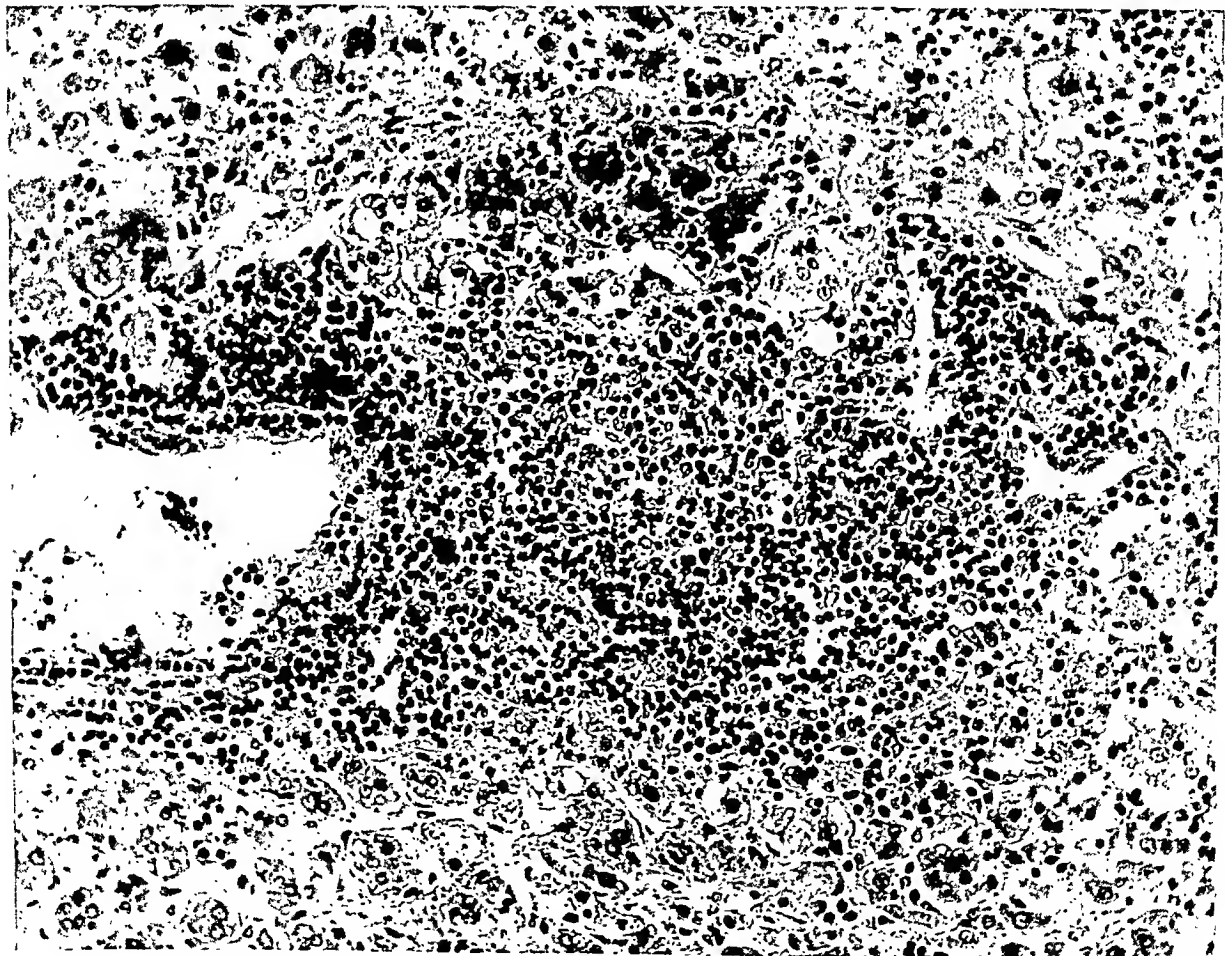
PLATE 102

FIG. 17. Section of vertebral bone marrow. A solitary trabeculum of necrotic bone crosses the center of the field. In it there are no osteocytes; the spaces. formerly filled with bone cells, are empty. The margins of the bone are rough and its ground substance shows linear longitudinal splitting and scaling. A few histiocytes, laden with thorotrast, lie in immediate apposition to this necrotic bone. On either side are fat cells, clusters of red blood cells, and small nests of both myeloblasts and erythroblasts. × 370.

FIG. 18. Section of adrenal, showing atrophy and fibrosis of the outer portion of the glomerular zone of the cortex. In this area of fibrous tissue there are small deposits of thorotrast. × 140.

FIG. 19. Kidney. A small lobular artery crosses the center of the field. The lumen of this vessel is abnormally wide and unevenly dilated. The intima is thin and composed of a single layer of endothelial cells supported by a poorly defined basement membrane. The media is composed of atrophic muscle fibers, each of which appears as a bare nucleus bordered by a narrow rim of scarcely stainable cytoplasm. There is little edema of the surrounding stroma. The glomerulus and tubules in this field show no abnormality. × 185.

FIG. 20. Lung, showing in the center of the field a medium-sized branch of a pulmonary artery. Muscle fibers, normally conspicuous in vessels of this order, are not recognizable. The wall is composed almost exclusively of hyalinized acellular fibrous tissue. The adventitia is made up of loosely arranged collagen. The adjacent alveoli are emphysematous. × 90.

17

1[

19

2[

MacMahon, Murphy, and Bates

Sarcoma of Liver Following Thorotrast
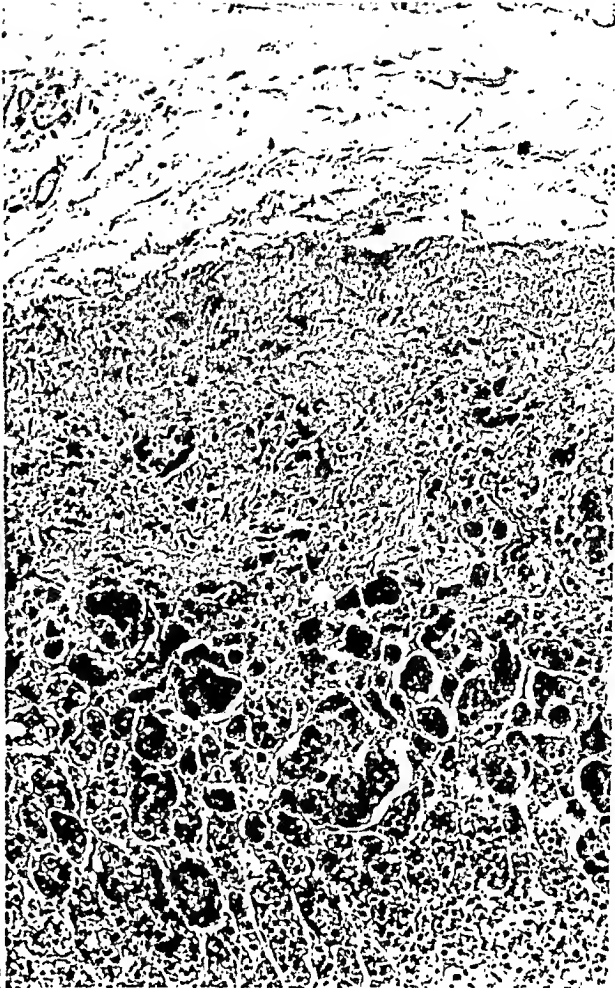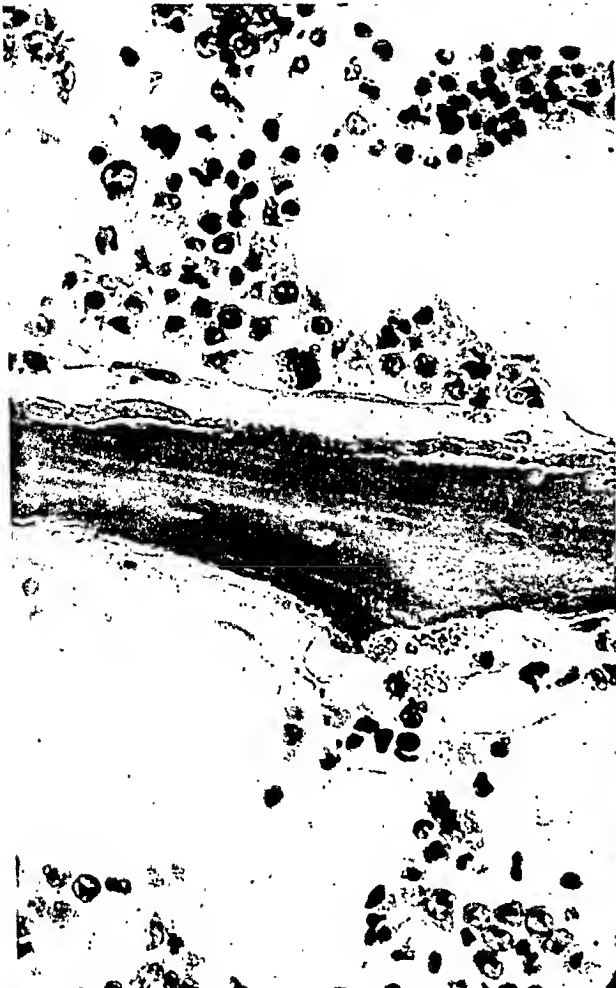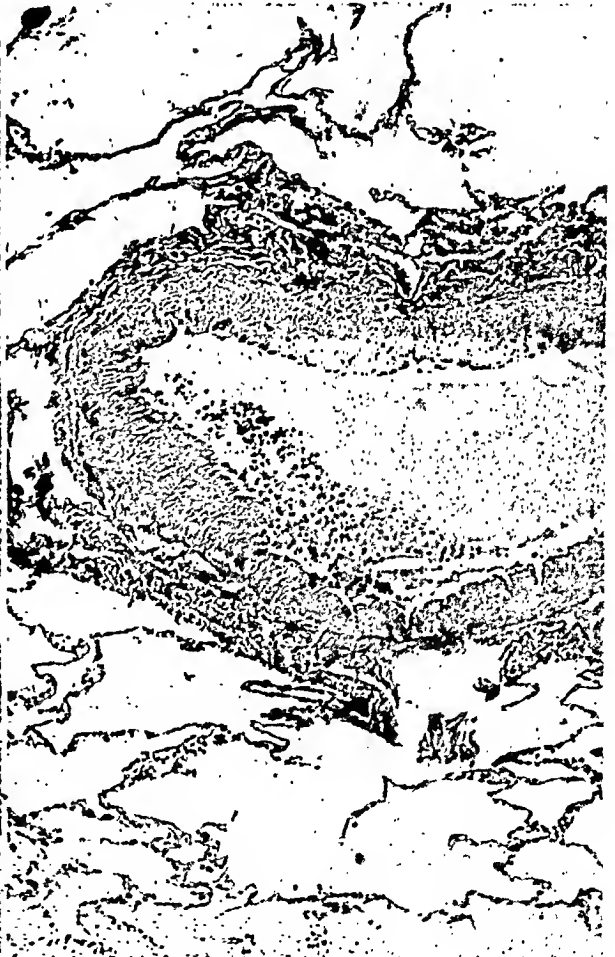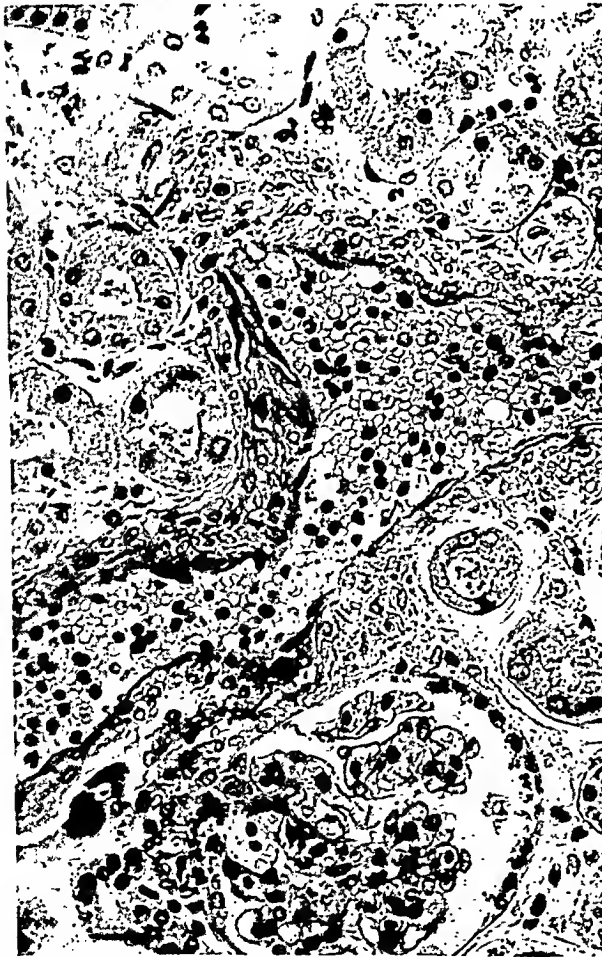
MYELOLIPOMA OF THE ADRENALS

Report of Seven Cases *

Horace K. Giffen, M.D.

(*From the Laboratories of the Youngstown Hospital Association, Youngstown 1, Ohio, and the Institute of Pathology, Western Reserve University, Cleveland 6, Ohio*)

After the incidental observation at autopsy of a moderately large fatty tumor in an adrenal gland, on gross examination thought to be a lipoma, which microscopically contained fat and the cellular components of bone marrow, attention was directed to the incidence and nature of this lesion.

Oberling [1] proposed the name myelolipoma, but it is questionable whether the condition is neoplastic. Gierke [2] believed that there is an immigration of elements of bone marrow at the time the epiblastic medullary tissue penetrates the mesoblastic cortex. Mieremet [3] also looked on the lesion as of embryonal origin. This would mean that it belongs in the general category of choristoma, an embryonal fault with neoplastic potentiality. Richardson [4] thought that this potentiality might occasionally be realized with formation of a true tumor. Saleeby,[5] generally in favor of ectopic or autochthonous origin, suggested the possibility that the lesion is a sort of benign bone marrow tumor, *i.e.*, a type of benign myeloma.

Arnold,[6] who reported the first case on record, looked on the lesion as an ectopia, a view shared by Kruse,[7] who found bone and bone marrow in the adrenal of a *Macacus rhesus* monkey, and shared also, apparently, by Oberling.[1]

Woolley,[8] who reported the presence of bone and bone marrow in a tuberculous adrenal, thought that metaplasia as the result of irritation was the most logical explanation. Newsam [9] found bony spicules and bone marrow, which he attributed to inflammation. Goldzieher [10] thought that the condition was due to metaplasia as the result of inflammation or cicatrization. Herzenberg [11] reported bone marrow in an accessory mass of cortical tissue attached to the hepatoduodenal ligament. She believed that there is an autochthonous development of bone marrow from capillaries, the causative insult being unknown. She compared this with the occurrence of myeloid tissue in chronic pancreatitis and in a rectus abdominis muscle. Gormsen [12] interpreted his first case in similar fashion.

Extramedullary hematopoiesis has been considered by Petri,[13] Brannan,[14] and by Gormsen.[12] Of particular interest is Gormsen's second case, a female, 53 years old, who had thrombocytopenic purpuric

hemorrhages in many parts of the body. Minimal extramedullary hematopoiesis was found in liver and spleen, but these had been irradiated therapeutically. There was tumor-like involvement of the lower half of the right, and of the whole of the left adrenal. Microscopically, there was almost complete replacement of the cortex by immature blood cells, especially of the erythrocyte series, with numerous mitotic figures. Moderate numbers of myeloid forms were present but there were no megakaryocytes. This extended into the periadrenal fat. Gormsen interpreted this as a reaction to the profound anemia, but was not clear whether it is an accentuation of a pre-existent condition or a new focus of extramedullary hematopoiesis. Brannan, however, indicated that extramedullary hematopoiesis occurs especially in adipose connective tissue, whereas ectopic bone marrow is rare without obvious relation to anemia.

Collins,[15] in 1932, collected from the literature 15 cases of bone marrow in the adrenal and added one of his own. He gave a good resumé of the literature and analysis of these cases. He divided the cases according to the method of Soós [16] into two types. In one type the tumor is yellowish orange and on microscopic examination is found to have predominance of adipose tissue with minimal myeloid elements and a larger proportion of erythroblastic elements. Tumors of the second type are dark red or reddish brown grossly, and microscopically show predominance of the cellular elements of the bone marrow, with the myeloid elements prominent and minimal erythroblastic elements. Collins found that 4 of the reported cases were of the first type with predominance of fat, and that 8 cases, plus one of his own, showed a highly cellular picture composed of marrow elements. In one of these no fat was seen. He stated that the average age of these 15 patients was 60.1 years and that the youngest reported was 42 years, until his own, who was 32. The tumor in his case was composed of adipose tissue, with islands of myeloid tissue, the entire nodule being surrounded by cortical cells. The cellular arrangement was characteristic of bone marrow with comparative paucity of erythroblastic elements, more "lymphocytes" than are usually seen in bone marrow, numerous premyelocytes and polymorphonuclear forms, and many megakaryocytes.

Reports of Cases

Case 1

A well developed white male, 40 years old, died abruptly as the result of rupture of a saccular aneurysm situated in the anterior communicating artery of the circle of Willis. There was little arteriosclero-

sis and no other aneurysms were found. The left adrenal weighed 45 gm. and was distorted by the presence of a spherical, soft, orange yellow, tumor-like mass (Figs. 1 to 4). The opposite adrenal was normal. Microscopically, the mass was composed of adipose tissue, moderately well vascularized, and islands of bone marrow cells. All stages of granulocyte formation were present as well as a few megakaryocytes. The mass was not encapsulated and was surrounded almost completely by adrenal cortex.

Case 2

A white female, 53 years of age, died at St. Alexis Hospital, Cleveland. The main cause of death was given by Dr. John L. Work as arteriolar nephrosclerosis and terminal bronchopneumonia. The right adrenal, weighing 12.5 gm., contained a small cortical nodule. The left adrenal, which weighed 12.0 gm., contained a spherical, dark red and yellowish gray nodule measuring 6 mm. in diameter (Figs. 5 to 7). This occupied the central zone of the adrenal but was surrounded by cortical tissue. Microscopically, this was an extremely vascular nodule of red bone marrow with a small amount of fat in the stroma. It was cellular, nonencapsulated, surrounded by cortex, and contained well defined erythropoietic foci and scattered myeloid elements.

Case 3

A white male, 59 years old, died at the University Hospitals of Cleveland (autopsy no. 7735), of myocardial infarction with stenosing coronary sclerosis. The right adrenal weighed 11.2, the left, 17 gm. In the left gland was a well demarcated, reddish brown nodule which measured 7 by 7 mm. The proportion of cortical tissue to medulla was markedly increased. Microscopically, the nodule in the left adrenal showed a richly cellular structure with a moderate amount of fat in the stroma. The picture was typical of red marrow, with megakaryocytes and all gradations of red and white cell formation in abundance (Fig. 11). The mass was without capsule, occupied the central part of the gland, and was surrounded by cortical tissue. Yellowish brown pigment granules were scattered in the stroma and in phagocytes.

Case 4

A colored female, 51 years of age, died of a cardiorenal syndrome, the heart weighing 825 gm. (autopsy no. 13246, Cleveland City Hospital). The left adrenal weighed 10, the right, 15 gm. In the latter there was an oval, fatty mass, 3 by 2 cm., surrounded by thinned cortex (Fig. 8). The center of the mass was composed of dark reddish brown, somewhat friable material, around which the periphery of the nodule

was yellowish gray. Microscopically, this mass was an extremely vascular, partially encapsulated, red marrow focus with fibro-adipose stroma rich in thin-walled blood vessels and sinusoids (Figs. 9 and 10). In the dense parts of the stroma were occasional spicules of bone. Cortical tissue almost completely surrounded the tissue mass and in some places the margin of the focus faded insensibly into an excessively vascular cortex. The marrow elements were pleomorphic, including all myeloid elements, megakaryocytes, and erythropoietic foci. Sicklemia was present.

Case 5

A white male, 76 years old, died of confluent bronchopneumonia (autopsy no. 11939, Cleveland City Hospital). He had arteriosclerosis and his heart weighed 500 gm. The right adrenal was normal. In the left adrenal was a moderately firm, spherical mass, 1 cm. in diameter, with a moist, yellowish gray cut surface, largely surrounded by cortex. Together the adrenals weighed 16 gm. Microscopically, the nodule had a fibro-adipose stroma with the connective tissue more prominent than in the usual lipoma. There was a large, dense, partially hyalinized zone of connective tissue in the center of the mass. The tissue was well vascularized, nonencapsulated, and in the stroma between fat cells there were a few young granulocytes and some erythroblastic cells in various stages of development. There were more erythropoietic than myeloid elements.

Case 6

A white male, 62 years of age, died of confluent bronchopneumonia, but with cardiorenal disease. The heart weighed 690 gm. (autopsy no. 6832, University Hospitals of Cleveland). The right adrenal weighed 9, and the left, 7.5 gm.; and in the left was a spherical, firm, homogeneous, grayish white nodule measuring 8 mm. in diameter. This was not encapsulated and occupied the central zone, being surrounded by cortex. There was no medulla at this site. Microscopically (Fig. 13), there were various erythropoietic elements and many blood sinusoids between the fat cells. Myeloid tissue elements were scanty. Many phagocytes were present; some were vacuolated and some contained yellowish brown pigment granules.

Case 7

A white female, 64 years old, died because of a massive ventral hernia with gangrene, perforations, and peritonitis (autopsy no. 14246, Cleveland City Hospital). Together, the adrenals weighed 15 gm. In the left was a large nodular tumor which was almost homogeneous, grayish yellow, and surrounded by cortex. It was ovoid and measured approximately 2 by 1.5 by 1 cm. Microscopically, it was

composed largely of fat with more vascularization than the usual lipoma (Fig. 12). There were numerous thin-walled blood sinusoids, some of which were large. In these were scattered foci of small cells with dense, often lobulated nuclei and pink, scanty cytoplasm. These showed the structure and cellular constituents of erythropoietic centers. Few myeloid elements were present. The tumor was not encapsulated.

DISCUSSION

These 7 cases were interpreted as showing foci of true bone marrow arranged in nodular or tumor-like form. In the course of study of this material many other adrenals were found which contained small amounts of adipose tissue. In some there were foci of cells foreign to the usual fat tissue, such as small and large nongranular mononuclear cells. In addition there were found four fatty tumor masses, with few or no myeloid elements, which were interpreted as lipomas. An additional similar case has been found recently in material at the Youngstown Hospitals. However, none of these fatty tumors was encapsulated and the vascularity and amount of stroma between the fat cells were more than is common in lipomas found elsewhere. The general structure was similar to that interpreted as fatty bone marrow except for paucity or absence of myeloid elements. Two hard tumor masses were encountered which showed a large deposit of brown pigment, calcium, and bone formation, but few or no myelocytic cells. These seem to have followed hemorrhage or infarction. In one of these the patient had multiple infarcts in other organs.

In all 7 cases there was some degree of fat in the stroma. Moreover, most or all of them were nearly or completely surrounded by adrenal cortical tissue. Cases 1, 5, 6, and 7 seem to fit into type I of Soós[16] with predominance of adipose tissue, minimal myeloid elements, and prominent erythropoietic tissues. Cases 2, 3, and 4 fit Soós' type II, showing a richly cellular, red marrow structure with prominent myeloid elements and relatively fewer erythroblastic elements. The average age of the patients was 57.9 years, the youngest being 40 years old. Four were males. All were observed incidentally at autopsy and the patients had no symptoms or signs attributable to the adrenal lesions. The division into the two types of Soós is somewhat artificial because these 7 cases show gradations of one type into the other. Even those lesions which might be called lipomas have more stroma, richer vascularization, and greater numbers of cells than is common in lipomas elsewhere.

Woolley[8] listed three hypotheses to account for heterotopic bone formation, as follows:

1. Embryonal rests of osteogenic tissue, misplaced during develop-

ment, which, with proper stimuli or changed physiologic conditions, produce bone or marrow.

2. Embolism of bone marrow cells by way of the blood stream.

3. Metaplasia, which holds that any cell under certain conditions may change its morphologic features and become physically and perhaps chemically like other cells originating from the same embryonal layer.

Woolley [8] called attention to the relationship between blood supply and bone formation, which many have investigated. He stated that ligation of renal vasa frequently results in bone or marrow formation, although in other organs only necrosis and calcification result. In the ordinary lipoma seen in surgical material the blood supply seems scanty and yet ossification is rare. In the adrenals containing these fatty nodules the blood supply seems abundant although the rate of flow may be slow. In my material the more cellular nodules were richly vascular, and in these the granulocytes were prominent. This perhaps fits in with the statement of Sabin [17] that widely dilated sinuses give abundant and slow blood flow, which brings the maturation factors in the right concentration for granulocytes between these vessels. She stated that for red cell formation collapsed capillaries with low oxygen tension are the most favorable and that the red elements are formed within the blood vessels.

The view that the lesions represent extramedullary hematopoiesis is not borne out by these cases, for in none of them was anemia a conspicuous feature.

The blood vessels are of mesoblastic origin and Kruse [7] suggested that in the course of development certain of the primitive cells become osteoblasts and hematoblasts instead of vascular endothelium.

SUMMARY

Seven examples of foci of bone marrow in the adrenal are reported, of which four represented yellow, and three red marrow. The features are similar to those reported in the literature. Although no positive conclusions as to origin can be drawn, the theory of Gormsen that they are derived from proliferation of the reticulo-endothelial cells of the blood sinuses is attractive. However, the stimulus which causes these cells to differentiate toward fat and bone marrow, in such a degree as to produce grossly visible nodules, has not been disclosed.

REFERENCES

1. Oberling, C. Les formations myélo-lipomateuses. *Bull. de l'Assoc. franç. p. l'étude du cancer*, 1929, 18, 234–246.

2. Gierke, E. Über Knochenmarksgewebe in der Nebenniere. *Beitr. z. path. Anat. u. z. allg. Path.*, 1905, Suppl. 7, pp. 311–325.

3. Mieremet, C. W. G. Ein aus den verschiedenen Elementen des Knochenmarks bestehender Tumor in der Nebenniere. *Centralbl. f. allg. Path. u. path. Anat.*, 1919–20, 30, 403–409.

4. Richardson, J. C. A tumour of the adrenal gland composed of the elements of bone marrow tissue. *Am. J. Cancer*, 1935, 25, 746–752.

5. Saleeby, E. R. Heterotopia of the bone marrow without apparent cause. *Am. J. Path.*, 1925, 1, 69–76.

6. Arnold, J. Ein Beitrag zu der feineren Struktur und dem Chemismus der Nebennieren. *Virchows Arch. f. path. Anat.*, 1866, 35, 64–107. Ueber die feinere Struktur der hämoglobinlosen und hämoglobinhaltigen Knochenmarkzellen. *Ibid.*, 1896, 144, 67–85.

7. Kruse, H. D. A case of bone formation in the medulla of the suprarenal gland. *Anat. Rec.*, 1924, 28, 289–294.

8. Woolley, P. G. Heteroplastic bone and bone-marrow formation associated with tuberculosis in the adrenal. *J. Lab. & Clin. Med.*, 1915–16, 1, 502–508.

9. Newsam, A. R. Calcification and bone formation in the adrenals. *Rhode Island M. J.*, 1924, 7, 35–38.

10. Goldzieher, M. A. The Adrenals: Their Physiology, Pathology and Diseases. The Macmillan Co., New York, 1929.

11. Herzenberg, H. Zur Frage der Heterotopie des Knochenmarkes. *Virchows Arch. f. path. Anat.*, 1922, 239, 145–152.

12. Gormsen, H. Über das Vorkommen von hämopoietischem Gewebe in der menschlichen Nebenniere. *Virchows Arch. f. path. Anat.*, 1943, 310, 369.

13. Petri, E. Ueber Blutzellherde im Fettgewebe des Erwachsenen und ihre Bedeutung für die Neubildung der weissen und roten Lymphknoten. *Virchows Arch. f. path. Anat.*, 1925, 258, 37–51.

14. Brannan, D. Extramedullary hematopoiesis in anemias. *Bull. Johns Hopkins Hosp.*, 1927, 41, 104–136.

15. Collins, D. C. Formation of bone marrow in the suprarenal gland. *Am. J. Path.*, 1932, 8, 97–105.

16. Soós, J. Zur Nebennierenpathologie. VI. Über Wucherungsherde roten und gelben Knochenmarks in der Nebenniere. *Beitr. z. path. Anat. u. z. allg. Path.*, 1930, 85, 611–618.

17. Sabin, F. R. Bone marrow. *Physiol. Rev.*, 1928, 8, 191–244.

[*Illustrations follow*]

DESCRIPTION OF PLATES

PLATE 103

FIG. 1. Case 1. Rounded, orange-yellow, fatty mass in the left adrenal weighing 45 gm. The tumor is surrounded by a thin rim of flattened cortex.

FIG. 2. Case 1. Margin of the tumor to show rim of cortex, absence of capsule and cellular marrow tissue in fat stroma. × 163.

FIG. 3. Case 1. Clumps of cells of hematopoietic series in vascular area with some fat. × 640.

FIG. 4. Case 1. Area showing two megakaryocytes and developmental forms of granulocytes as well as a hematopoietic focus at the lower left. × 350.

FIG. 5. Case 2. In this adrenal (left) the nodule is dark red, homogeneous, surrounded by cortex but without a capsule. It is in an adrenal which is otherwise normal.
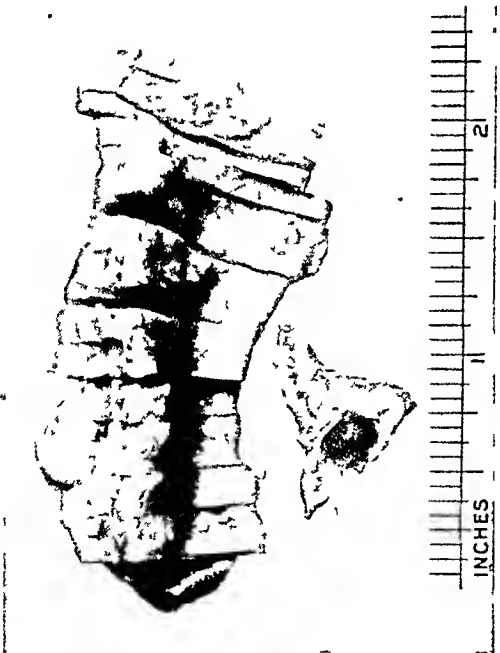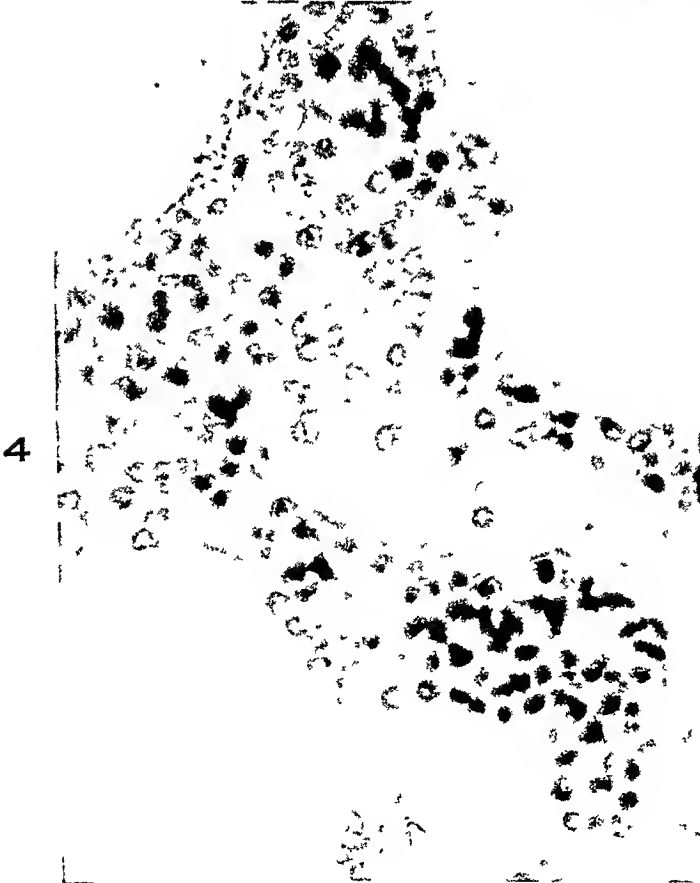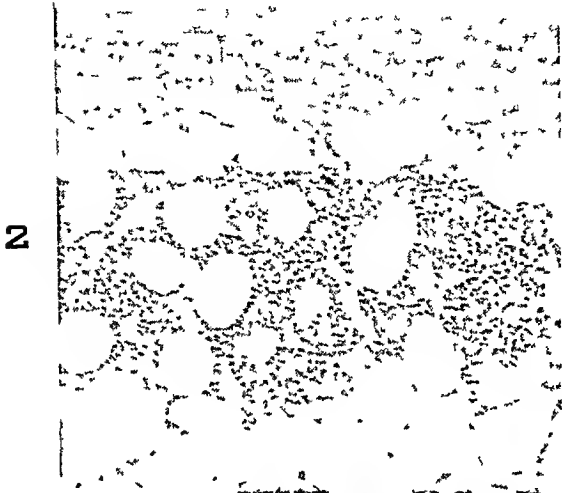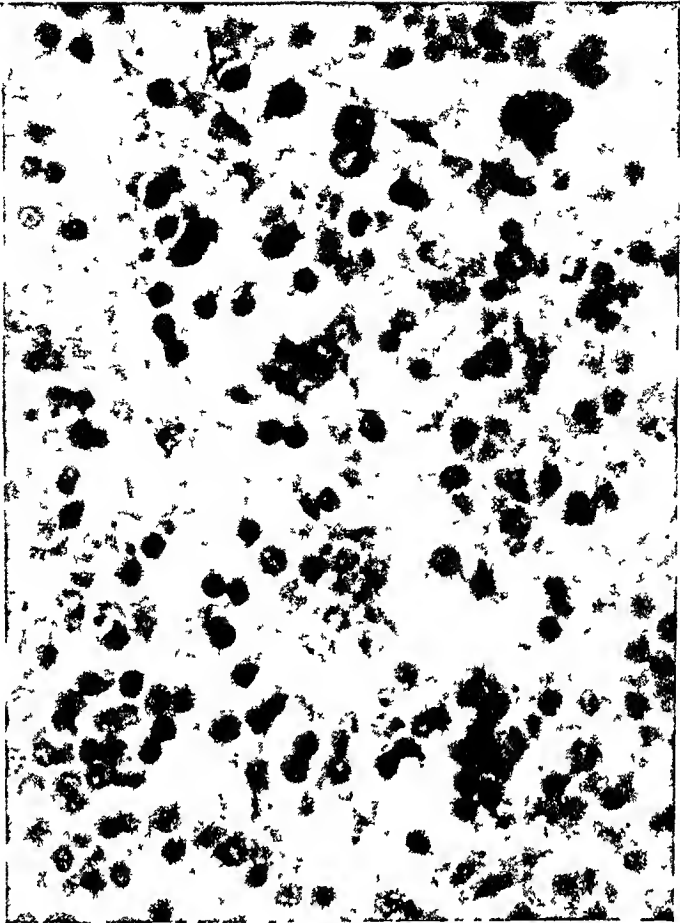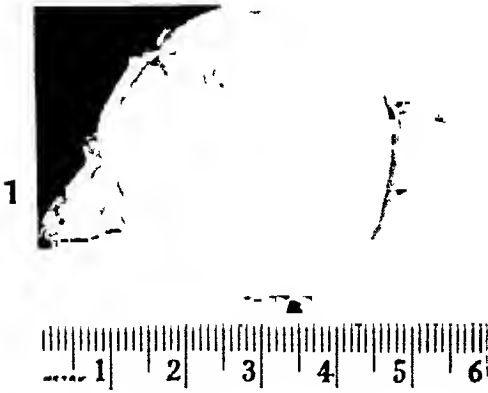
Giffen

Myelolipoma of the Adrenals

PLATE 104

FIG. 6. Case 2. An area in the adrenal tumor in which hematopoiesis predominates but developmental forms of red and white cells are represented. × 540.

FIG 7. Case 2. Higher power view of the same lesion as in Figure 6, to show megakaryocytes and all other elements of red bone marrow. × 640.

FIG 8. Case 4. Photograph of the right adrenal showing poorly defined masses scattered in the cut surface. The tumors were similar microscopically, with a fatty stroma, focal fibrosis, calcification, and ossification. The masses are extremely vascular with cellular constituents pleomorphic.

FIG 9. Case 4. Low power magnification to show the moderately fibrous zone with little fat and abundant hematopoietic activity. × 213.

6
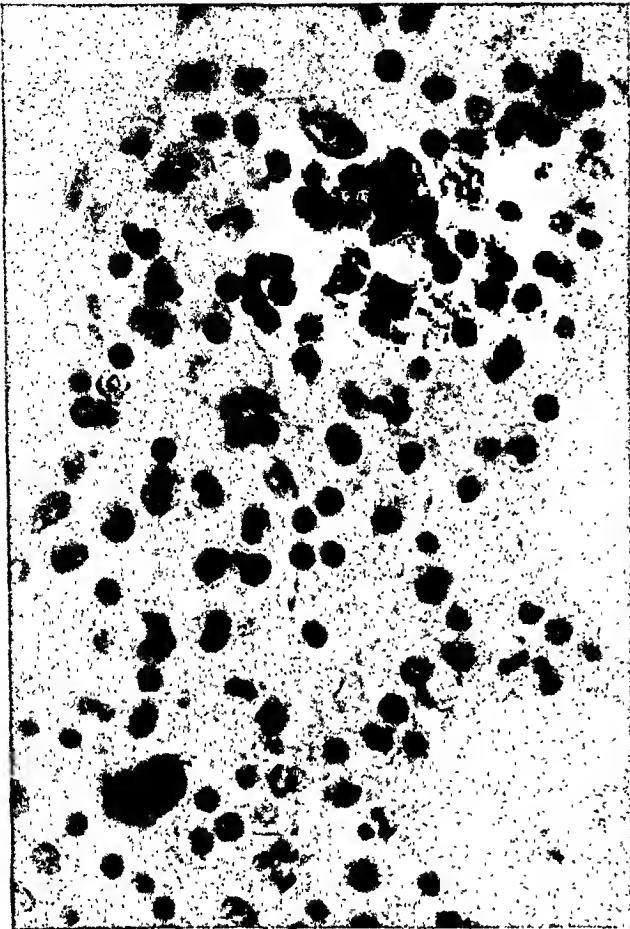
7
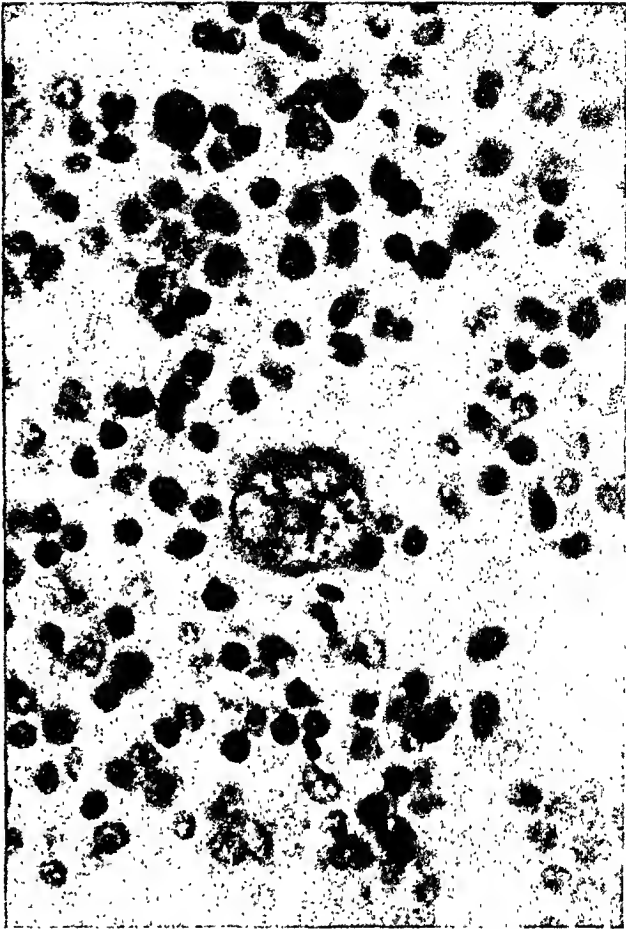
8
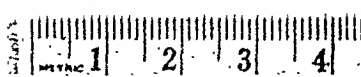
9

Giffen

Myelolipoma of the Adrenals

FIG. 10. Case 4. Pleomorphic zone of the adrenal tumor, in which there are atypical megakaryocytes and scattered granulocytes. The dark cell in lower left center is multinucleated with overlapping nuclei. $\times$ 615.

FIG. 11. Case 3. Photomicrograph to show the outer part of the red marrow mass with fat in the stroma. Both granulocytic and erythrocytic cells are abundant, but no megakaryocytes are seen. $\times$ 164.

FIG. 12. Case 7. Photomicrograph of the grayish yellow mass in the cortex. Stroma is fatty with moderate vascularization, and foci of erythropoiesis are scattered in the vascular channels. $\times$ 141.

FIG. 13. Case 6. Photomicrograph of tissue from the firm grayish white nodule in the left adrenal, showing a cellular focus in an extremely vascular fatty stroma. Here are seen mostly erythropoietic cells in dilated blood sinuses. There are also scattered cells of the granulocytic series in the stroma but no megakaryocytes are found. $\times$ 665.

10
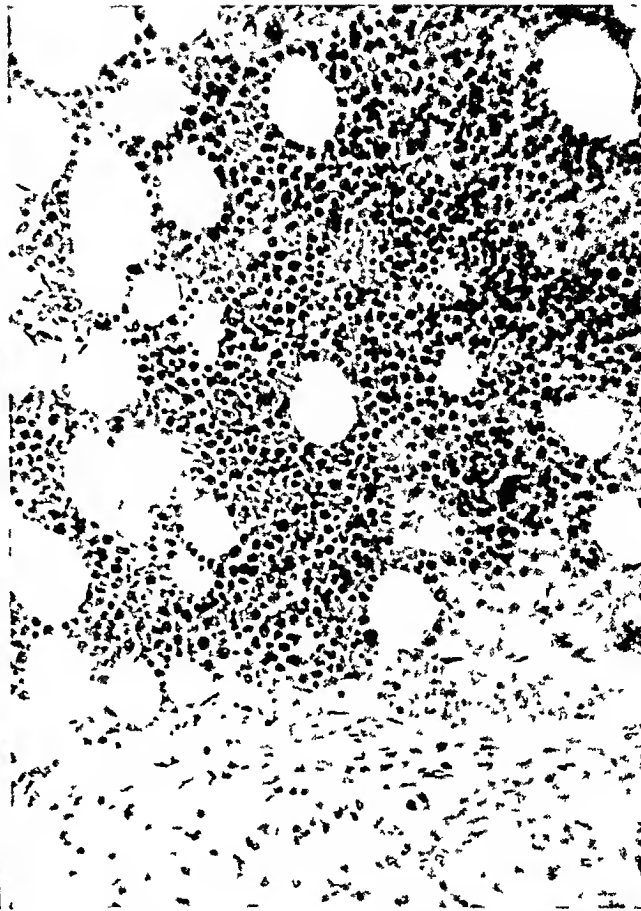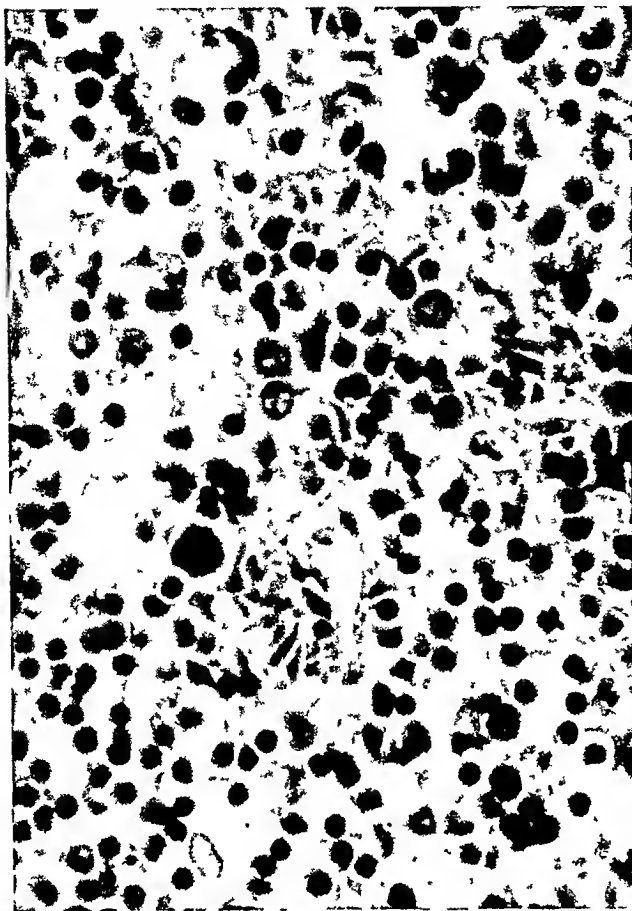
12
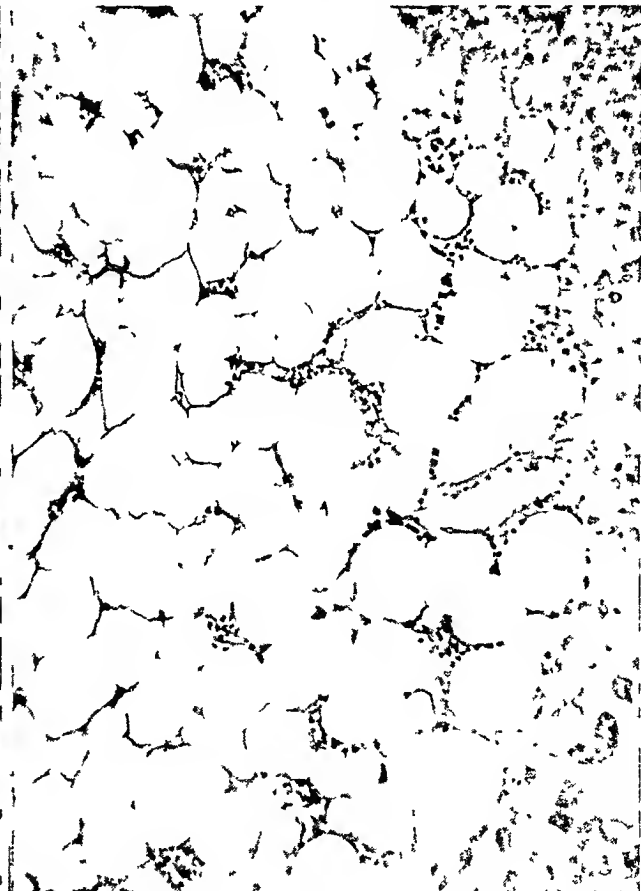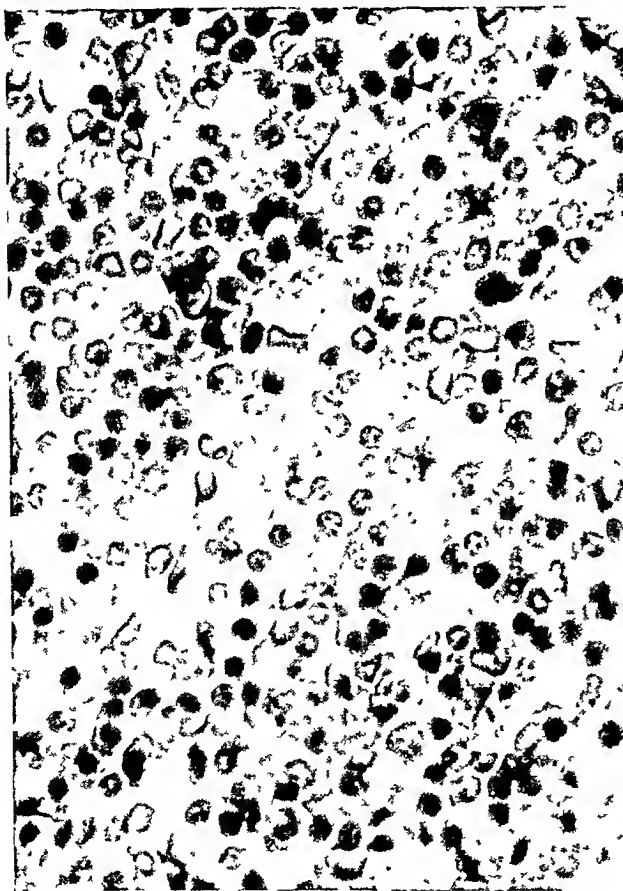
Giffen

Myelolipoma of the Adrenals

OBSERVATIONS ON TYZZER'S DISEASE IN MICE *

Capt. Fred L. Rights, Sn.C., A.U.S., Elizabeth B. Jackson, Civilian Technician, and Lt. Col. Joseph E. Smadel, M.C., A.U.S.

(From the Virus Division of the First Medical General Laboratory, U. S. Army, and the Division of Virus and Rickettsial Diseases, Army Medical School, Washington 12, D.C.)

A spontaneous disease of Japanese waltzing mice characterized by large focal inflammatory lesions of the liver was noted and described by Tyzzer [1] in 1917. His excellent photomicrographs illustrate the long, banded, bacilliform organisms found in affected cells of the liver and intestinal mucosa. Although the organism associated with the disease could not be cultivated, it was regarded as the etiological agent and called *Bacillus piliformis*, solely on morphological grounds. Experimental transmission of the disease to normal waltzing mice was accomplished with some regularity by contact with diseased animals or by the ingestion or intravenous injection of very large doses of infected tissues. However, the appearance of the disease in various control groups indicated its general presence throughout these transmission experiments. Ordinary laboratory animals were not susceptible, but the disease was later found by Tyzzer [2] in certain inbred strains of mice being used for cancer studies.

A disease apparently identical with that described by Tyzzer was encountered in a British strain of Swiss mice during the functional activities of the First Medical General Laboratory, U. S. Army, in England. The present report reviews briefly the natural infection and the characteristics of the associated organism; in addition, it describes for the first time the regular and consistent experimental transmission of this disease in mice and other laboratory animals, and the cultivation of the organism in tissue cultures.

Materials and Methods

Laboratory Animals. The laboratory animals employed in these studies, including the Swiss mice in which the disease was enzootic, were obtained from the British Agricultural Experiment Station at Compton, England. The farm strain of mice was an inbred stock which had originated from the cross breeding of albino with wild mice.

Culture Media. The basal medium employed in the present work was standard beef heart infusion broth prepared from the dehydrated powder † or from fresh material.[3] This broth base contained 0.8 per

cent NaCl and 1 per cent of neopeptone or proteose peptone. Various combinations of blood, serum, glucose, ascorbic acid, and thioglycollate were added to this medium for enrichment. The Löffler's, Löwenstein-Jensen's, and Sabouraud's media were the standard preparations used in diagnostic bacteriology.[8]

Cultural Methods. Cultures were incubated at 37.5°C. except in a few instances when parallel cultures were carried at room temperature (20 to 23°C.). An atmosphere of 10 to 12 per cent CO_2 was obtained by the candle jar method. A McIntosh-Fildes' jar was used to obtain anaerobiosis which was checked by a methylene blue-alkaline glucose control tube.

Tissue Cultures. Agar slope tissue cultures were prepared according to the method described by Zinsser, Wei, and Fitzpatrick.[4] The minced tissues of either 10-day-old chick embryos or of 15 to 20-day-old mouse embryos served as a source of cells. A 10 per cent suspension of infected mouse brain was added to the embryonic tissue and the mixture was spread on serum agar slopes; the tubes were stoppered with rubber plugs and incubated aerobically at 37°C. for 5 to 10 days.

Histological Methods. Tissue specimens for microscopical examination were fixed in Zenker's formalin solution; paraffin sections were stained with hematoxylin and eosin, by Giemsa's method, or by Weigert's modification of Gram's stain.

EXPERIMENTAL FINDINGS

The Natural Infection

Outbreaks of the natural infection occurred in the mouse colony in the late winter and early spring of 2 successive years. The epizootic was associated in each instance with the overcrowding of breeding stocks. Mice which succumbed to the disease showed only vague, indefinite signs of illness consisting of a ruffled coat, an emaciated appearance, and occasionally a mild diarrhea. The autopsy findings, which were essentially the same as those described by Tyzzer, revealed a liver mottled by large (1 to 2 mm.), hard, yellow, inflammatory lesions with punctate necrotic centers. These were visible through the abdominal wall after the skin had been reflected. No other macroscopic lesions were observed consistently. Histological examinations of the hepatic lesions showed that the characteristic structure consisted of a central area of necrosis frequently surrounded by a zone of infiltrating polymorphonuclear cells with a peripheral area in which the parenchymatous cells had undergone some necrobiotic changes (Fig. 1). In this marginal zone, hepatic cells were seen frequently with their cytoplasm

filled with the organisms associated with the disease. These organisms were regularly demonstrated microscopically in stained impression smears of infected tissue and in smears of suspensions of ground tissue. They appeared as gram-negative, long, thin, nonbranching, multiple, banded rods which were often fusiform (Fig. 4). Although pleomorphic bulbous structures of the type described by Tyzzer were seen occasionally, we were not convinced that they warranted a classification of the organism as a member of the spore-forming group. Darkfield examinations of infected tissue suspensions failed to demonstrate motility.

Experimental Transmission of the Disease in Mice

Attempts were made to transmit the spontaneous disease of Swiss mice to apparently healthy mice of the same stock with suspensions of affected liver tissue. In one series of experiments, groups of 10 mice were either forcibly fed or inoculated by the intraperitoneal, intravenous, or subcutaneous route with such suspensions. No significant increase in incidence of hepatic disease was found in the treated groups during a period of 40 days' observation, when compared with the untreated control group. In contrast, the intracerebral inoculation of mice with suspensions of affected liver tissue resulted in each of three instances in the production of an acute encephalitis in the treated animals. The experimental disease was transmissible by intracerebral injection of 10 per cent suspensions of brain tissue from infected mice. One strain, called ML-7, was maintained in this manner through 100 serial transfers; the work with this strain provided most of the material for the present study. This method of producing an experimental encephalitis is important for the further investigation of the disease since transmission by other methods has not been satisfactorily consistent, and the etiological agent does not grow on ordinary culture media.

The Experimental Encephalitis in Mice

Swiss mice inoculated intracerebrally with suspensions of infected tissue usually died with signs of disease of the central nervous system between the third and the eighth day after injection. Affected animals developed increased irritability and paralysis of one or more limbs followed by convulsions and death, all in the course of a few hours. Neither the brain nor the viscera of mice dying with encephalitis showed macroscopic lesions. Histological examination of affected brains revealed areas of complete liquefaction necrosis which were sometimes surrounded by a moderate infiltration of polymorphonuclear cells (Fig. 2). The characteristic banded organisms were frequently

seen at the periphery of the necrotic foci; these were generally in an intracellular position (Fig. 5).

The Susceptibility of Other Laboratory Animals

Farm mice, white rats, rabbits, and hamsters were tested for their susceptibility to the experimental encephalitis. Farm mice were as susceptible to this infection by the intracerebral route as were the Swiss mice from which the agent originally had been recovered. Rats, rabbits, and hamsters also developed encephalitis when injected intracerebrally with 10 per cent suspensions of brain tissue infected with the agent of mouse liver disease. Microscopical examination of the brains of these animals revealed the presence of lesions similar in all respects to those found in experimentally infected mice. Figure 3 illustrates a lesion in the brain of an infected hamster.

Cultivation of the Piliformis Organism

The cultivation of the piliformis organism on cell-free media has been unsuccessful in our hands. In addition to the ordinary bacteriological culture media, a number of enriched media were employed under various environmental conditions. Certain of these media were of the type ordinarily employed for the cultivation of pleuropneumonia organisms. Preparations of such inoculated fluid and semisolid media were stained by Giemsa's as well as Gram's method for microscopical examination. A summary of the work is presented in Table I.

Some success was achieved, however, when the cultivation of this agent was attempted in serum agar slope, tissue cultures. Tissue cultures were examined microscopically for evidence of growth of the typical organisms and were tested for the presence of bacterial contaminants by the routine inoculation of blood agar plates. In addition, the pathogenicity of these tissue cultures was determined by the intracerebral inoculation of mice. Only slightly encouraging results were obtained when the cultivation of the piliformis organism was attempted on tissues from minced chick embryos. In three separate series of experiments, a moderate number of morphologically typical organisms were present in the primary culture and these proved to be virulent for mice; subcultures, however, contained only a few organisms and were completely avirulent.

In contrast, the use of embryonic mouse tissue in the agar slope cultures met with more success. Cultivation in this medium resulted in an abundant growth of the piliformis organisms which appeared typical of those seen in stained smears of material from the natural or experi-

mental lesions in mice. Materials from the original and first subculture were virulent for mice, and microscopical examination of the brains of the animals which succumbed showed that they contained piliformis organisms. Although the second, third, and fourth subcultures were rich in organisms, they failed to induce obvious disease in mice. Identical results were obtained on another occasion in a similar series of cultures.

TABLE I

Culture Media Used in Attempted Cultivation of the Piliformis Organism

Base	Medium	Enrichments	Ph	Atmospheric environment	Period observed
					(days)
Beef heart infusion	2% agar	8% sheep blood	7.2–7.6	Air	10
Beef heart infusion	2% agar	8% sheep blood	7.2–7.6	10% CO_2	10
Beef heart infusion	2% agar	8% sheep blood	7.2–7.6	Anaerobic	10
Beef heart infusion	2% broth	0.05% thioglycollate	7.2–7.6	Air	10
Beef heart infusion	2% broth	0.05% thioglycollate + 10% horse serum	7.2–7.6	Air	10
Beef heart infusion	Broth	0.2% dextrose + 20% horse serum + 0.05% ascorbic acid	7.2–7.6	Air	10
Beef heart infusion	Broth	0.2% dextrose + 20% horse serum + 0.5% ascorbic acid	7.2–7.6	Anaerobic	10
Beef heart infusion	Broth	5% rabbit blood	7.8–8.0	Air	14
Beef heart infusion	2% agar	5% rabbit blood	7.8–8.0	Air (moist chamber)	14
Beef heart infusion	Broth	30% horse serum	7.8–8.0	Air	14*
Beef heart infusion	2% agar	30% horse serum	7.8–8.0	Air (moist chamber)	14
Beef heart infusion	Broth	20% rabbit serum	7.8–8.0	Air	14
Beef heart infusion	1.5% agar	25% rabbit serum	7.8–8.0	Air (moist chamber)	14*
Löwenstein-Jensen	None			Air-sealed caps	21
Löwenstein-Jensen	0.05% thioglycollate			Air-sealed caps	21
Sabouraud's*	2% dextrose				10

* At least 2 or more blind passages were made in this medium.
All media were inoculated with 10% suspensions of mouse brain which were known to contain the agent since they subsequently killed normal mice when injected intracerebrally.

In every case the organisms grown in the tissue cultures were not ordinary bacterial contaminants, since they failed to multiply on blood agar plates. In summary, the piliformis agent grew abundantly in embryonic mouse tissues cultures but lost its virulence for mice after a few such serial passages.

Effect of Storage on the Agent of Tyzzer's Disease

The infectivity of mouse brain or liver tissue, either in the form of 10 per cent suspensions or as blocks of organs, was destroyed by storage in the frozen state for longer than 2 weeks. However, the agent

was regularly recoverable from infected tissues stored at $-20°$ or $-70°C.$ for only a few days.

DISCUSSION

Overcrowding undoubtedly provided one of the important factors in the explosive epizootics of liver disease which occurred among our mice. On each occasion of an outbreak, it was found that the mice, after receipt from the breeder, had been kept for some time under unsatisfactory conditions. The disease returned to a sporadic state when animals in subsequent shipments were provided with adequate quarters. The marginal war diet given mice may have contributed to their susceptibility, but it was probably not a factor in the sharp outbreaks since essentially the same foodstuffs were fed by both the breeder and us. It would appear that the disease was enzootic in the British stock mice and that it flared up when conditions were adverse, a common epidemiological observation. Some evidence for its frequent presence was the finding on a number of occasions of structures which appeared typical of the piliformis organism in stained smears of spleens of apparently normal mice of the same stock. Tyzzer likewise regarded the disease as one which occurred enzootically.

The part the piliformis organism plays in the etiology of mouse liver disease has not been clearly established. In our experience, as in Tyzzer's, this organism was always present in material infectious for mice. Indeed, the incubation period of the experimental disease was usually inversely proportional to the number of organisms seen in infected mouse tissue employed as inoculum. However, in certain instances obvious illness was not induced in mice by inocula rich in the organisms; notably, in those experiments with piliformis grown for several passages on tissue cultures.

These observations may indicate that the piliformis organism is the sole etiological agent of the fatal encephalitis in mice but that it is a fastidious organism which rapidly loses its virulence when grown in tissue culture. On the other hand, they may indicate that piliformis and an associated agent are responsible for the mouse disease and that piliformis grows in tissue culture but the concomitant agent does not. Serological studies, which unfortunately we were unable to complete, should contribute to an understanding of this problem. Van Rooyen [5] has commented on the morphological similarity of *Bacillus piliformis* and *Streptobacillus moniliformis,* but further analogies between these organisms are lacking. Neither is there weighty evidence which suggests that the agent of Tyzzer's disease is a member of the pleuropneumonia group of organisms.[6]

SUMMARY

Tyzzer's disease (epizootic hepatitis) of mice can be transmitted experimentally to mice, rats, hamsters, and rabbits by the intracerebral inoculation of infectious material. The experimental cerebral lesions, like those occurring in the livers of naturally infected mice, consist of focal areas of liquefaction necrosis surrounded by an acute inflammatory infiltration and contain many of the bacteria-like piliformis organisms. The piliformis organism is consistently associated with the natural and experimental disease in animals, and can be grown in tissue culture, but has not yet been cultivated in media devoid of living cells.

REFERENCES

1. Tyzzer, E. E. A fatal disease of the Japanese waltzing mouse caused by a spore-bearing bacillus (*Bacillus piliformis*, n. sp.). *J. M. Research*, 1917–18, **37**, 307–338.
2. Tyzzer, E. E., cited by Dingle, J. H. Infectious Diseases of Mice. In: Snell, G. D. (ed.) Biology of the Laboratory Mouse. The Blakiston Co., Philadelphia, 1941, p. 418.
3. Mackie, T. J., and McCartney, J. E. Handbook of Practical Bacteriology. E. & S. Livingstone Ltd., Edinburgh, 1945, ed. 7, pp. 78–158.
4. Zinsser, H., Wei, H., and Fitzpatrick, F. Nouvelles méthodes de culture de Rickettsiae du typhus à propos de la production de vaccins. *Compt. rend. Soc. de biol.*, 1938, **127**, 229–232.
5. Van Rooyen, C. E. The biology, pathogenesis and classification of *Streptobacillus moniliformis*. *J. Path. & Bact.*, 1936, **43**, 455–472.
6. Sabin, A. B. The filtrable microörganisms of the pleuropneumonia group. *Bact. Rev.*, 1941, **5**, 1–66.

[*Illustrations follow*]

DESCRIPTION OF PLATE

PLATE 106

FIG. 1. Photomicrograph illustrating a typical lesion in the liver of a naturally infected mouse. There is an area of liquefaction necrosis surrounded by a zone of infiltrating cells. Hematoxylin and eosin stain. × 100. (Neg. no. 93334.)

FIG. 2. Photomicrograph illustrating acute necrosis in the brain of a mouse with experimental encephalitis. Hematoxylin and eosin stain. × 100. (Neg. no. 93157.)

FIG. 3. Photomicrograph showing the edge of a zone of necrosis in the brain of a hamster injected intracerebrally. Polymorphonuclear cells are found in the disorganized adjacent area. Hematoxylin and eosin stain. × 600. (Neg. no 93339.)

FIG. 4. Photomicrograph of piliformis organisms found in the hepatic lesion of a mouse with the natural disease. Of note is the characteristic banded appearance of the organisms. Gram's stain (Weigert's method). × 1350. (Neg no. 93155.)

FIG. 5. Photomicrograph of a section of the brain of a mouse dying from experimental encephalitis. Clusters of the organisms are seen in the remnants of cells and lying loose in the tissue. Gram's stain. × 1360. (Neg. no. 93340.)
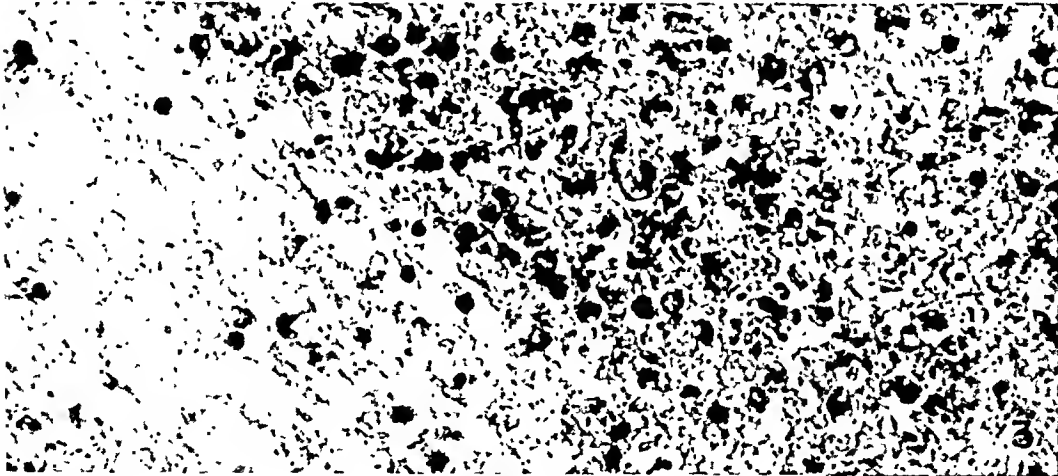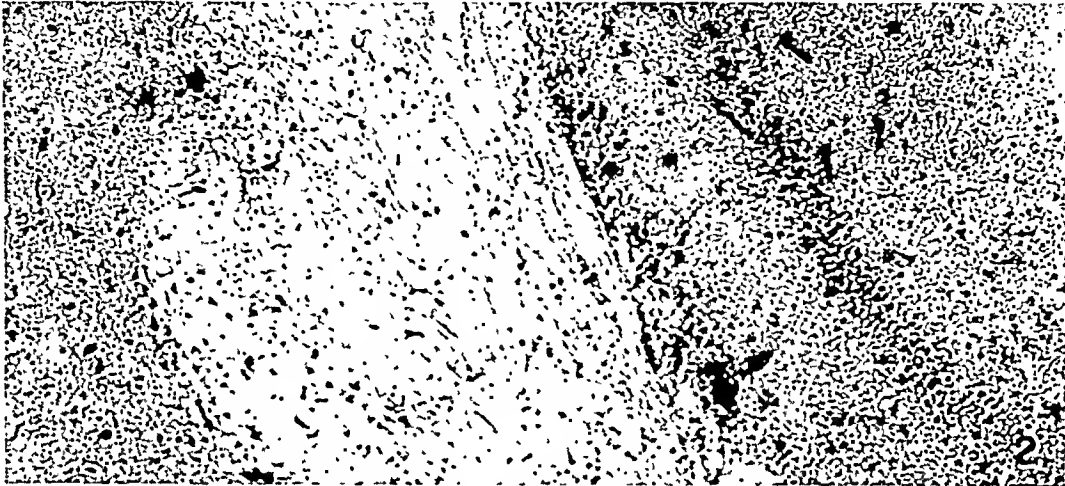
THE HISTOPATHOLOGY OF ACUTE MUMPS ORCHITIS [*]

EDWARD A. GALL, M.D.

(From Bethesda Hospital, Cincinnati 6, Ohio)

Orchitis developing in association with mumps is universally recognized as a prevalent concomitant of this disease.[1] Frequency of occurrence varies with age and with each epidemic. With the exception of encephalomyelitis,[2] it is probably the most serious complication of the mumps syndrome. The possibility of residual atrophy with sterility lends particular interest to the malady.[1]

Despite the interest and attention which have been directed to the disease, there is comparatively little knowledge regarding the underlying pathologic lesion or its mode of development. This is not surprising in view of the low mortality rate, the self-limited character of the process, and the infrequency with which surgical therapy has been invoked. Stolz,[3] Reuscher,[4] Hall,[5] and Malassez[6] have described the late lesions appearing in chronic orchitis. Only in the case described by Manca[7] and in the two cases recorded by Smith[8] is there information pertaining to acute mumps orchitis in the human being. Findlay and Clarke[9] observed the testicular lesions in monkeys with experimental mumps.

In the course of a previously reported epidemic of mumps among military personnel,[10] the procedure of orchidotomy as suggested by Wesselhoeft and Vose[11] was carried out by surgeons upon approximately 85 patients with orchitis. The proponents of the method believed that incision through the tunica albuginea testis would serve to relieve intratesticular tension and thus avoid the sequelae of necrosis and atrophy. In carrying out this procedure it was noted that parenchymatous substance immediately bulged through the incised capsule. Accordingly, minute fragments were removed for histologic study.

Seventy-five such fragments, none of which exceeded 0.4 cm. in diameter, were found to be suitable for histologic study. Since this form of therapy was considered to be indicated only early in the course of the disease, none of the material received represented the lesion beyond the fifth day. The majority of the patients were orchidotomized within 48 hours of the onset of symptoms. One additional patient with mumps complicated by bilateral orchitis and femoral thrombophlebitis succumbed to massive pulmonary embolism 11 days after the onset of orchitis. Material from both testes obtained at autopsy in this case

and the 75 specimens obtained for biopsy serve as the basis for this report.

In most of the fragments studied, a strip of tunica albuginea was included with the parenchymatous tissue. In 18 instances there were portions of extratesticular and extra-epididymal appendages included. With one fragment accidentally, and in the testes from the necropsied case, there were portions of the epididymis available for study. Zenker's fluid was utilized as a fixative for the autopsy material and 10 per cent formalin for the biopsy tissue. Sections were cut in paraffin and stained with hematoxylin and eosin. Connective tissue stains and examination for bacteria and inclusion bodies served no additional purpose.

Histologic Observations

Despite the fact that all but one of the specimens were obtained within 5 days of the onset, there was nevertheless a wide variation in the extent and intensity of involvement. In view of the rapidity with which the disease reached its peak of clinical intensity, this variation appears understandable. The telescoping of range of transition within a brief period would naturally permit a rapid change in the nature of the lesion. Under such circumstances it was impossible to establish a consistent correlation between clinical duration and the character of the lesion. In part, this was due to the variation in intensity of the disease in different patients. Moreover, it seemed evident also that scrotal pain was not an infallible criterion of the onset of orchitis, for in certain instances it resulted from epididymitis or inflammation of extratesticular appendages. Such a condition, masking or mimicking orchitis as it might, precludes the acceptance of pain as an index of either duration or intensity.

On histologic grounds, however, there seemed reasonable evidence of a divisible developmental trend. Consistent microscopic features appeared to justify the assumption that the acute lesion in the testis was susceptible to subdivision into four fairly distinctive phases.

Testis

In 6 specimens of testis it was impossible to detect any abnormality whatever (Fig. 1). There was normal structural arrangement and no evidence of either inflammation or edema. In each of these patients symptoms had been present for 24 hours or less, and it is presumed that they may have arisen either from involvement of the extratesticular appendages or from intratesticular foci not included in the sections.

The earliest abnormality recognized in 31 preparations was interstitial edema. This was manifested in the tunica albuginea by separa-

tion of connective tissue fibers and in the parenchyma by broadened interstices, fraying and floating of connective tissue elements, precipitated fluid, and separation of seminiferous tubules (Fig. 2).

Germinal epithelium exhibited minor degenerative changes such as cytoplasmic swelling, increased depth of nuclear staining, and desquamation of surface epithelium into the lumen. Spermatogenesis, however, appeared to proceed in unimpeded fashion.

The next (second) phase, exemplified by 10 specimens, was characterized by definite, but relatively slight, reactive change. There was a considerable degree of vascular dilatation and engorgement, particularly in the deep portion of the tunica albuginea and in the loose fibrillar zone intermediate between capsule and parenchyma. Small numbers of lymphocytes were clustered about capsular blood vessels. Interstitial edema persisted in the parenchymatous region and, in addition, arterioles showed mural thickening. This was in part the result of swelling of smooth muscle components and in part due to proliferation as evidenced by increased numbers of smooth muscle nuclei. Here, as in the capsule, there was a scant, loose, perivascular aggregation of lymphocytes (Fig. 3). Germinal epithelium showed little additional degeneration, although many of the tubular lumina contained precipitated fluid in addition to desquamated elements.

Fifteen specimens showed progression to a more advanced (third) stage. Perivascular lymphocytic collars in the capsule were wider and more thickly populated and there was now a sprinkled infiltration of the edematous capsule as a whole by similar cells. As before, in the deep portion of the capsule and in immediate subcapsular stroma, the reaction was more pronounced. Not only were lymphocytes more numerous here, but there was also an irregular hemorrhagic extravasation. Many arterioles showed mural thickening (a single instance of arteritis with thrombosis was encountered), but as a rule evidence of unquestionable intrinsic vascular damage was lacking. There was no arterial necrosis. Polymorphonuclear leukocytes, though present, were sparse, but there was a rather pronounced membrane-like deposit of fibrin particularly evident in the immediate subcapsular region.

Within the parenchyma itself the lesion was distinctly spotty (Fig. 6). Many areas retained the appearance described in the earlier stages. Here and there the perivascular lymphocytic reaction was more intense and spread in an irregular manner within the intertubular tissues. Clusters of Leydig cells were not primarily affected but were often partially obscured by the advancing exudate. In some areas lymphocytes were so numerous that they completely filled, in a closely packed manner, the space intervening between tubules (Figs. 4 and 5).

·Where the exudate was less pronounced, perivascular aggregations of lymphocytes attained considerable depth. There were an associated scanty fibrin deposit and comparatively few polymorphonuclear leukocytes, the latter exhibiting no focal concentration. There were also interstitial hemorrhages which, though usually small, were in some instances widespread and coalescent. In such circumstances encompassed tubules were sharply outlined by the brightly staining mass of erythrocytes. Neither thrombosis nor necrosis appeared in the interstitial tissues.

During this phase, for the first time, inflammatory elements were noted within the seminiferous tubules. This reaction was also focal and appeared but rarely in areas free from interstitial exudate. Its composition differed from that of the interstitial process in that the exudate was composed preponderantly of polymorphonuclear leukocytes. Among these cells were a few phagocytic macrophages. The germinal epithelium exhibited progressive degeneration with pyknosis and cytoplasmic fragmentation. Ultimately all of the epithelium in an affected tubule was dislodged from its mural attachment, spermatogenesis ceased, and an agglomeration of fragmented cells, débris, polymorphonuclear leukocytes, and phagocytes formed in the lumen. Only Sertoli cells remained attached to the lamina propria of the tubule. These cells, though frayed, did not at this time appear intrinsically damaged. These supporting elements seemed to coalesce and form a narrow tessellated syncytium lining the tubule. At the same time the lamina propria showed a segmental arcuate thickening which eventually involved the entire circumference. This apparently was initiated in those portions adjacent to interstitial blood vessels, the adventitia of which contained reactive exudates. The thickening seemed to result partially from fibroblastic proliferation, partly from infiltration by inflammatory exudate, and partly by apparent fibrosis of residual Sertoli elements (Fig. 5). The intramural exudate, comprised initially of lymphocytes, became polymorphonuclear in character as the intraluminal lesion progressed.

It could not be determined with certainty whether or not this change occurred along the entire longitudinal extent of a tubule. From the appearance of those sectioned sagittally and the larger sections available from the necropsy, it appeared that this was indeed the case. In other words, though focal and spotty with regard to the testis as a whole, individual tubules were involved throughout their entire extent.

The most advanced acute lesion (fourth stage) in this study was observed in 13 specimens. Although focal distribution was maintained,

there was evidence of a tendency for the involvement to become diffuse with few of the parenchymatous structures remaining uninvolved (Fig. 7). Capsular changes had not progressed beyond those previously described. The interstitial tissue, however, was completely filled with densely packed lymphocytes, among which relatively small numbers of polymorphonuclear leukocytes and macrophages were evident. In some cases a thick intertubular fibrin deposit was noted and in others there were many foci of hemorrhage. The lumina of the tubules now contained no viable germinal epithelium, nor was the Sertoli cell syncytium apparent. Instead, the tubules were distended and plugged by dense masses of polymorphonuclear leukocytes which lay enmeshed within a delicate fibrin network (Fig. 8). Interspersed among these cells were fewer but variable numbers of macrophages, lymphocytes, and fragments of cellular débris. The lamina propria, though thickened and infiltrated by leukocytes, was intact and there was no evidence of destruction of sustentative elements. Despite the degree of damage, it would seem from the nature and extent of the changes that complete atrophy of the testis would be an unusual sequela. That permanent damage may result focally, however, was shown in the later stages observed in the patient who was studied by necropsy.

Material from both testes was examined in the fatal case. The lesions differed in several respects from those noted above, probably as the result of the duration of the process (11 days). They seemed to be more intense than in the surgically procured specimens. Interstitial tissue in all areas (the sections were sagittal and included the entire gonad) was markedly edematous and contained large foci of hemorrhage, a considerable fibrin deposit, and a heavy infiltration of lymphocytes and macrophages. Despite the intensity of this lesion, there were very few polymorphonuclear leukocytes. Many of the phagocytes contained vacuoles and hyperchromatic detritus. The capsular reaction was identical with that described above. Several large groups of tubules showed retention of normal epithelium which was proliferating actively (Fig. 9). The great majority, however, suffered from a variety of changes ranging from desquamation with heavy mural infiltration of lymphocytes and plasma cells to complete cessation of active inflammation with organization of the tubules. This was manifested by increased thickness of the lamina propria and collagenization of both the lamina and the Sertoli remnants attached to the wall. In tubules so affected, permanent atrophy undoubtedly resulted. Between the two extremes cited there were many tubules, the lumina of which were filled with coagulated débris and many lymphocytes and laden

phagocytes. It is interesting that in none of the tubules in this case were polymorphonuclear leukocytes so prominent a feature as in the earlier and more acute lesions described above.

Testicular and Epididymal Appendages

Eighteen examples of testicular and epididymal appendages were studied. All showed some evidence of inflammatory infiltration which was not unlike that seen in the tunica albuginea of the testis. There were edema and congestion of variable degree. Some capillary channels were widely dilated and pool-like in appearance. There was a variable amount of perivascular lymphocytic aggregation which was spotty in distribution. In one case with a history of scrotal pain intermittent for months preceding the attack of mumps, a large number of eosinophils were found interspersed among the lymphocytes. This probably represented a mumps-induced exacerbation of a nonspecific epididymitis. It was the only instance in which granulocytes were found in the lesion of the appendage. Surface epithelial elements and those lining the pinched off downpouchings were wholly intact.

Epididymis

Sections from three epididymides were available (two of these were obtained at autopsy). The appearance of the connective tissue was identical with that described in the testicular capsule and the appendages. In the main there was a focal lymphocytic exudate associated with relatively little fibrin deposit and a rather marked vascular engorgement. The infiltration about the ducts, however, was intense, consisting of closely packed lymphocytes clustered about the tubules and often completely filling the interductal spaces (Fig. 10). The epithelial cells lining the ducts were intact and apparently unaffected. The lumina were usually unremarkable, but in some instances contained masses of desquamated epithelium and débris and even a few small clumps of polymorphonuclear leukocytes. It is believed that these represented products of the orchitic process which had been swept up into the epididymis.

DISCUSSION

Both Wolbach (in Smith[8]) and Manca[7] described lesions which, with but little variation, resembled the process as it was manifested in the present series of cases. Wolbach noted the focal character of the exudate in the tunica albuginea, the edema, small hemorrhages, and perivascular exudates. He found, however, that the reactive cells were not lymphocytes but "endothelial leukocytes" and polymorphonuclear

leukocytes. This was also the case in the parenchymatous process. Here the contrast between intratubular and extratubular exudates was not as striking as in the acute cases in the present series. He found polymorphonuclear cells predominating in both regions. Manca examined both testes in a case which was autopsied several days after the onset of mumps orchitis. In his material, too, there was close similarity to the observations made in the present series. Lymphocytes appeared in great numbers in the interstitial area but polymorphonuclear leukocytes were increased in the more advanced foci. The fibrin deposit seemed to be greater than was apparent in the current group. The epididymitis was identical in appearance with that described above.

The pathogenesis of the lesion remains somewhat obscure. Inclusion bodies similar to those described by Johnson and Goodpasture [12] were not recognized in Giemsa-stained preparations of several of our specimens. It would seem that whatever the cause there was initially a mild but widely distributed vascular injury manifested by edema, congestion, and perivascular lymphocytic clustering. It is possible, as suggested by Wesselhoeft and Vose,[2,11] that with this as an initiating impetus and the limited expansibility of the tough tunica albuginea as a concomitant, the degenerative and suppurative changes were altogether secondary in nature. In other words, the lesion is presumed to represent a combination of mild inflammatory response to the mumps virus and pressure necrosis inherent upon the architecture of the testis. The specific necrotizing effect upon the germinal epithelium in contradistinction to the stimulating effect upon the supporting structures may be attributed to the more highly specialized development of the former elements. Epithelial cells would, under such conditions, be expected to exhibit less resistance to disturbance of nutrition than would sustentative tissues. Another factor which may in part explain the disproportionately greater amount of intratubular reaction is the failure of drainage from tubules plugged by fibrin and desquamated epithelium.

In the absence of evidence of widespread organization of the damaged testis it would not seem likely that persistent significant clinical stigmata might be anticipated. Certainly, complete atrophy should not be expected in the face of the spotty distribution of the lesion. It is probable that scattered tubules might fail to regenerate and, as noted in the fatal case, become hyalinized. It is not unusual to find islands of hyalinized tubules in otherwise normal testes examined incidentally at necropsy. In view of the collagenization observed at 11 days, it is reasonable to assume that these may represent in some instances the residua of previous attacks of mumps. In the unusual case of orchitis, in

which the disease is fulminating and diffuse, it is probable that complete atrophy and, in the event of bilateral involvement, sterility may result. Unfortunately, save for the rare case subjected to delayed surgical intervention or autopsy, interpretations of atrophy recorded in the literature are based upon conclusions reached by clinical examination. Since relative size and consistency of the gonads are used as criteria, interpretative accuracy must be considered equivocal.

SUMMARY

Testicular and epididymal tissue obtained from 76 cases of acute mumps orchitis (75 for biopsy and one at autopsy) have served as the basis for this study. In all cases testicular tissue was available. In 18 cases there was material from the appendages and in 2 (3 specimens) from the epididymis. Material was obtained during the first 5 days of symptoms in all but one case (11 days).

Considerable variation was evident in the character and extent of the lesions in the testis but it seemed that a developmental trend could be detected. From early edema and a scant perivascular lymphocytic exudate the process progressed to a diffuse lymphocytic infiltration of the interstitial tissue with focal hemorrhage and pronounced destruction of germinal epithelium, with plugging of the tubules by epithelial débris, fibrin, and polymorphonuclear leukocytes. The intratubular lesion remained focal in most instances but in a few cases every tubule in a given section was involved. Evidence of collagenization was elicited only in the one late case.

Inflammation of the testicular appendages and the epididymis remained confined to the connective tissue elements and, with a single exception, was wholly lymphocytic in character. Epithelial elements were unaffected in these structures.

REFERENCES

1. Stengel, A., Jr. Mumps orchitis. *Am. J. M. Sc.*, 1936, 191, 340–356.
2. Wesselhoeft, C. Mumps: Its Glandular and Neurologic Manifestations. In: Virus and Rickettsial Diseases. Harvard University Press, Cambridge, 1940, pp. 309–348.
3. Stolz, A. Ueber Totalnekrose des Hodens bei Mumps. *Mitt. a. d. Grenzgeb. d. Med. u. Chir.*, 1901, 7, 392–406.
4. Reuscher, K. Anatomischer Beitrag zum Ausgang der Mumpsorchitis. *Ztschr. f. urol. Chir.*, 1926–27, 21, 249–259.
5. Hall, H. C. Über Hodenatrophie nach Parotitis epidemica. *Virchows Arch. f. path. Anat.*, 1912, 207, 188–194.
6. Malassez. Cited by Comby, T. In: Twentieth Century Practice. W. Wood & Co., New York, 1898, 13, p. 559.

7. Manca, C. Über die Mumpsorchitis. *Virchows Arch. f. path. Anat.,* 1932, 285, 426–442.

8. Smith, G. G. Two cases of orchitis due to mumps treated by operation. *Boston M. & S. J.,* 1912, 167, 323–325.

9. Findlay, G. M., and Clarke, L. P. The experimental production of mumps in monkeys. *Brit. J. Exper. Path.,* 1934, 15, 309–313.

10. McGuinness, A. C., and Gall, E. A. Mumps at Army camps in 1943. *War Med.,* 1944, 5, 95–104.

11. Wesselhoeft, C., and Vose, S. N. Surgical treatment of severe orchitis in mumps. *New England J. Med.,* 1942, 227, 277–280.

12. Johnson, C. D., and Goodpasture, E. W. The histopathology of experimental mumps in the monkey, *Macacus rhesus. Am. J. Path.,* 1936, 12, 495–510.

[*Illustrations follow*]

DESCRIPTION OF PLATES

PLATE 107

FIG. 1. Testicular parenchyma, as observed in 6 cases with scrotal pain and mumps. No abnormality is detected. Tubules show active spermatogenesis and are closely approximated with little intervening stroma. × 200.

FIG. 2. The earliest perceptible change in mumps orchitis. There is pronounced interstitial edema with separation of tubules and little or no cellular exudate. Mild degenerative changes have occurred in the germinal epithelium. × 200.

FIG. 3. A second phase of the lesion, showing in addition to interstitial edema a sprinkled infiltration of lymphocytes having predilection for a perivascular distribution. There is some increase in intratubular degenerative change. × 200.

FIG. 4. The interstitial exudate has increased in intensity. It is almost wholly lymphocytic in character. Spermatogenesis has ceased and a scant intratubular exudate of polymorphonuclear leukocytes and macrophages has appeared. × 200.

Gall

Histopathology of Acute Mumps Orchitis

FIG. 5. In addition to a lymphocytic exudate and hemorrhage in the interstitial tissue, there is now a rather dense polymorphonuclear exudate in the tubules. The lamina propria of the tubule appears thickened and Sertoli cell remnants have fused to form a frayed syncytium. $\times$ 200.

FIG. 6. The parenchymatous reaction is shown to be spotty. $\times$ 40.

FIG. 7. The process now appears more diffuse and there is suppuration within many tubules. $\times$ 40.

FIG. 8. Seminiferous tubules filled completely with a polymorphonuclear exudate. The sole residue of the pre-existing tubule is the moderately thickened lamina propria. Interstitial stroma shows edema, hemorrhage, and lymphocytic infiltration. $\times$ 200.
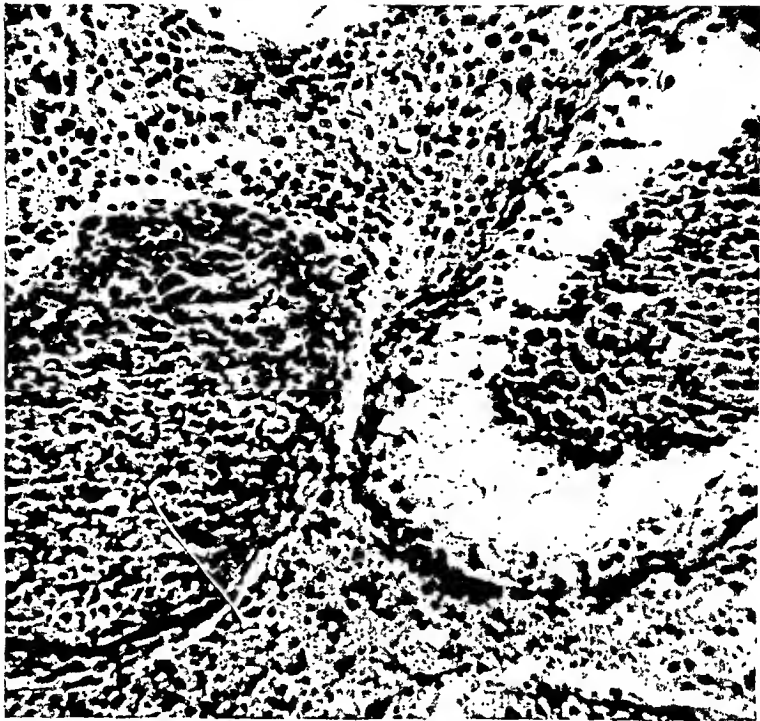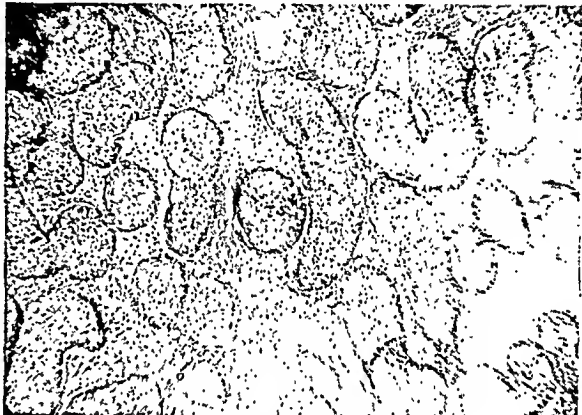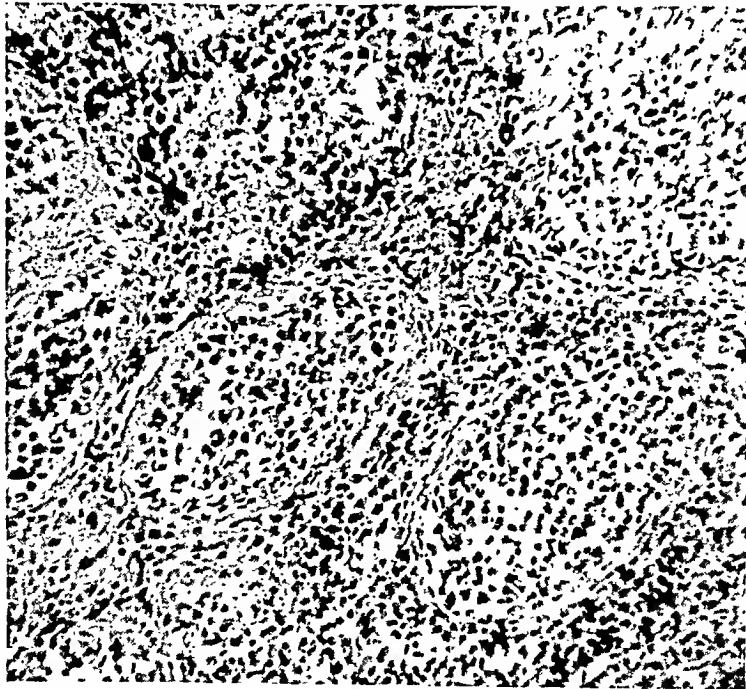
Plate 108

Gall

Histopathology of Acute Mumps Orchitis

PLATE 109

FIG. 9. An area from a testis 11 days after the onset of mumps orchitis. An actively functioning seminiferous tubule contrasts with the surrounding intense inflammatory reaction. Adjacent tubules are the seat of severe degenerative and inflammatory changes. $\times$ 200.

FIG. 10. Epididymis in mumps orchitis. There is interstitial edema and a rather pronounced lymphocytic infiltrate. Tubular epithelium is preserved but the lumina contain an exudate presumably swept up from the severe orchitic process. $\times$ 200.
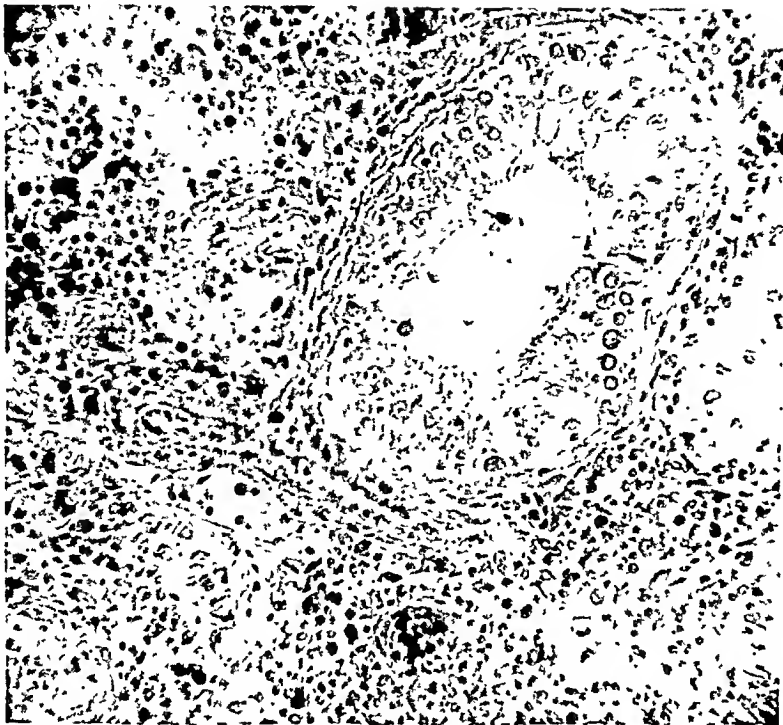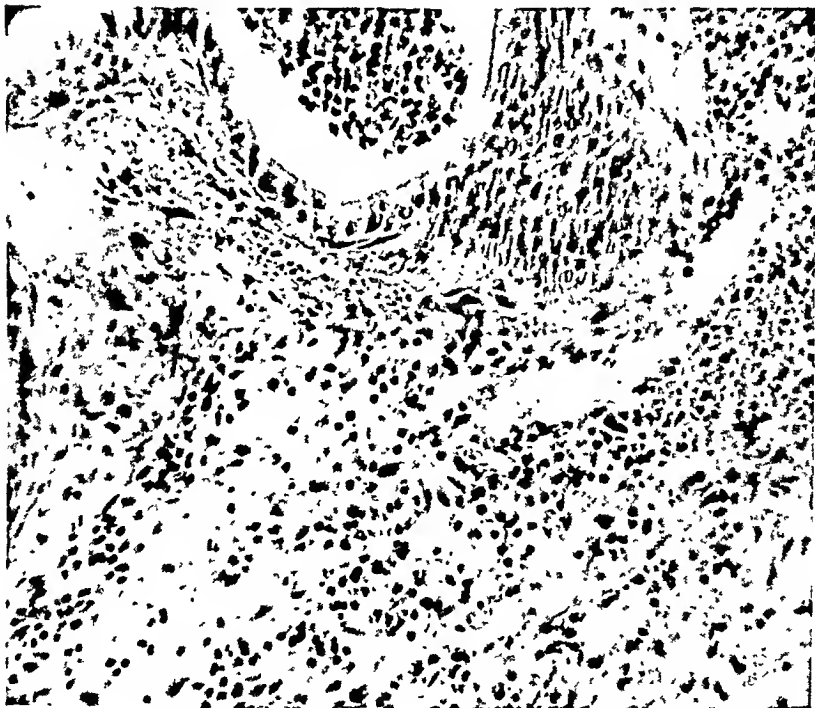
Gall

Histopathology of Acute Mumps Orchitis

LYMPHOGRANULOMA VENEREUM

A Histologic Study of the Primary Lesion, Bubonulus, and Lymph Nodes in Cases Proved by Isolation of the Virus *

Walter H. Sheldon, M.D., and Albert Heyman, M.D.

(From the Departments of Pathology and Medicine (Clinic for Genitoinfectious Diseases), Grady Memorial Hospital and Emory University School of Medicine, Atlanta, Ga.)

The histologic picture of lymphogranuloma venereum has received little attention since few pathologists have had an opportunity to examine proved lesions of this disease. The diagnosis of this infection is often difficult. Clinical criteria are uncertain; skin and complement-fixation tests are of limited value since they do not necessarily indicate active infection but remain positive long after the disease has subsided. The isolation and identification of the virus is not a practical diagnostic procedure since it is technically difficult. Only recently has it been shown that the complement-fixation reaction can be of value in the diagnosis of active lymphogranuloma venereum.[1]

An extensive investigation of the clinical and laboratory aspects of lymphogranuloma venereum has been completed in this clinic[2] and a number of lesions were excised for histologic study. The majority of the lesions described in this report were proved to be lymphogranuloma venereum by the isolation and identification of the virus. We have not found in the literature any similar histologic studies based on proved material.

LITERATURE

Since several complete reviews on lymphogranuloma venereum have appeared in the recent literature, only a brief summary of the histologic findings will be given here. Textbooks of pathology[3-7] and monographs[8-11] describe the lesions in lymph nodes as follows: The early lesion consists of small masses of epithelioid cells with multinucleated giant cells scattered throughout the gland. These are similar to those seen in tuberculosis or syphilis. The center of the lesion becomes necrotic and contains polymorphonuclear leukocytes, but no true caseation or calcification is evident. Stellate-shaped abscesses are formed and are surrounded by a narrow layer of epithelioid cells which are arranged in palisade formation. Small intracytoplasmic chromatophil bodies of varying shapes are found within large mononuclear cells in the débris of the abscess. These are called "Gamna bodies" and

were thought to be inclusions associated with the lymphogranuloma virus. The lymph node shows hyperplasia and diffuse inflammatory cellular infiltration. This consists of plasma cells, neutrophilic and eosinophilic polymorphonuclear leukocytes, and basophils, in addition to large mononuclear cells, multinucleated giant cells, and lymphocytes. Fibroblasts proliferate from the thickened capsule into the lymph node. The blood vessels are dilated and may show periarteritis or obliterating endarteritis.

Pund, Greenblatt, and Huie[12] stressed the marked "peritubular" infiltration with plasma cells and lymphocytes, and the dilated lymphatics. Kornblith[13] emphasized that the general architecture of the node is preserved and that the secondary follicles are prominent. The reticulum is intact except in the necrotic center of the lesions. Necrosis is prominent in early lesions while later stages show more fibrosis. After 5 to 6 months the characteristic lesions tend to disappear and only small foci of epithelioid cells surrounded by plasma cells remain in the cortex of the node.

The histologic picture of the primary lesion of lymphogranuloma venereum is rarely described. Hansmann[14] has studied the primary lesion in two patients. These lesions were extragenital and were located on the back of the hand and in the upper dorsal region, respectively. The inflammatory process involved the corium and subcutaneous tissue with small foci of large mononuclear cells, plasma cells, lymphocytes, and occasional giant cells. One of these foci showed central necrosis with many giant cells and resembled tuberculosis. Necrosis, however, was absent in most of the other lesions which were reminiscent of Boeck's sarcoid. The process in the primary lesions was identical with that in the regional lymph nodes. Hansmann also reviewed the descriptions of the primary lesion by Bory[15] and Phylactos[16] who described small ulcerations on the penis with a marked inflammatory cellular infiltration, consisting chiefly of plasma cells and few polymorphonuclear leukocytes. The same cells surrounded the lymphatics. Many newly formed blood vessels were seen and the walls of some of the smaller vessels were thickened. Stannus[11] also reviewed the literature and described epidermal changes with edema of the malpighian and prickle-cell layer and sometimes ulceration. The underlying connective tissue was infiltrated by plasma cells, lymphocytes, swollen connective tissue cells, and cells with several nuclei. The endothelium of the congested blood vessels was swollen but there was little perivascular infiltration. In more advanced cases, however, the process showed characteristic micro-abscesses resembling those found in lymph

nodes. D'Aunoy and von Haam [8] distinguished a herpetiform and chancroidal type. The latter showed necrosis of the epithelium and connective tissue, and dilated lymphatics filled with large endothelial cells. The herpetiform lesion displayed hyperplasia of the granular layer of the epidermis and infiltration of the rete malpighii by round cells and polymorphonuclear leukocytes. An excellent illustration of the primary lesion was shown by Ash and Spitz [3] who described a non-specific granuloma occasionally containing many eosinophils.

We were unable to find a histologic description of the bubonulus of lymphogranuloma venereum. The lesion is a circumscribed swelling, several centimeters in diameter, which occasionally is found in the prepuce or skin of the penis. It was first described by Brandt.[17]

Method of Study

This report is part of an investigation of the clinical and laboratory aspects of lymphogranuloma venereum in which 28 patients were proved to have this disease by isolation and identification of the lymphogranuloma virus.[18,19] Each of these patients was carefully examined and subjected to a large number of tests. These procedures included skin tests, lymphogranuloma complement-fixation tests, serum protein determinations, formol-gel reactions, biopsies, autoinoculations, cultures, smears, darkfield examinations, and serologic tests for syphilis. The virus was isolated from each of these patients by inoculation of bubo material intracerebrally in mice and into the yolk sac of developing chick embryos. These viruses produced symptoms of meningo-encephalitis in mice and were pathogenic for the developing chick embryo. Smears of the infected mouse brains and yolk sacs revealed elementary bodies of the type found in the psittacosis-lymphogranuloma group of infectious agents. Positive identification of each of these strains as lymphogranuloma virus was made on the basis of their morphologic features, pathogenicity, tissue tropism, sulfonamide susceptibility, and neutralization tests. Smears and cultures of the aspirated material from each patient were negative for the Ducrey bacillus and other bacteria. Darkfield examination of the primary lesions of these patients was negative for *Treponema pallidum*.

The primary lesion of lymphogranuloma venereum was excised from 3 patients subsequently proved to have this disease by isolation and identification of the virus from the associated inguinal bubo. Additional primary lesions were obtained from 4 other patients from whom the virus was not isolated. The clinical and laboratory findings in these 4 patients were consistent with the diagnosis of lymphogranuloma

venereum, and the histologic picture of these four lesions was identical with that seen in patients proved to have the disease. All seven lesions were located on the penile shaft or prepuce.

Inguinal lymph nodes were excised for histologic examination from 2 patients (nos. 171 and 187) and the virus was isolated from these specimens. A third inguinal lymph node was excised from a patient (no. 1) who developed a recurrence of the disease with swelling of the originally infected lymph nodes immediately after a Frei test. The lymphogranuloma virus was isolated from this node. Six months before, the patient had shown clinical and laboratory evidence of acute lymphogranuloma venereum which subsided with treatment. A fourth inguinal lymph node (patient no. 161) also was examined. The virus of lymphogranuloma venereum had been isolated by aspiration of this node 9 months previously. The infection had subsided with treatment and showed no clinical or laboratory signs of activity.

A typical bubonulus located in the prepuce was likewise removed for histologic study and the virus was isolated from this specimen as well as from the associated inguinal lymph node. All of the patients in this series were Negro males.

After excision the tissue from the primary lesion was fixed immediately in Regaud's fluid except for the specimens from 2 unproved cases which were fixed in Zenker's fluid. The inguinal lymph nodes and the bubonulus were sectioned under sterile precautions, and one-half of each specimen was used for virus studies. Slices of the remaining tissue were fixed in Regaud's and Zenker's fluids.

Serial sections were made from all tissues. Material fixed in Regaud's fluid was stained by Giemsa's method recommended by Wolbach and Pinkerton.[20] Zenker-fixed tissue was stained with phloxine and methylene blue. Consecutive sections of the lymph nodes and of the bubonulus were stained with Mallory's aniline blue, Wilder's reticulum, and Weigert's elastic tissue stains. These stains were used only on occasional sections of the primary lesion.

HISTOLOGIC OBSERVATIONS

The Primary Lesion

The primary lesion was a shallow ulcer in the penile skin or mucosa not more than 2 or 3 mm. deep (Fig. 1). Its fundus consisted of necrotic tissue and was covered by an exudate of fibrin, cellular débris, and some neutrophilic polymorphonuclear leukocytes. Occasional microorganisms, both cocci and bacilli, were found in the superficial layers of the exudate.

An area of dense inflammatory cellular infiltration surrounded the

ulcer. This area was ill defined and merged with the surrounding tissue. Large mononuclear cells predominated but some neutrophilic polymorphonuclear leukocytes also were seen. Plasma cells were numerous toward the periphery of the lesion and a few lymphocytes were present also.

The large mononuclear cells were often aligned along the fundus of the ulcer in palisade arrangement. These cells formed nodules which originated in the adventitia of small blood vessels (Fig. 2). The large mononuclear cells had proliferated and involved not only the surrounding tissue but progressed toward the lumen of the vessel which was gradually compressed and eventually disappeared (Fig. 3). Small solid granulomata were thus formed. The occlusion of the vessel was not associated with significant endothelial proliferation. At this stage the pattern of the vessel wall was visible only with the reticulum stain. Necrosis with polymorphonuclear leukocytes occurred in the centers of these granulomata which then formed small abscesses (Fig. 4).

These granulomata or abscesses might coalesce and this led to the formation of the ulcer. The fundus of the ulcer showed the same architecture and cytologic detail as the granulomata in which abscesses had formed (Fig. 5). Thus the cavity of the ulcer was the necrotic core of the granuloma, the contents of which had been for the most part evacuated. The fundus represented the peripheral zone of large mononuclear cells of the granuloma.

The large mononuclear cells were of the usual type with vesicular nuclei and ample cytoplasm. Multinucleated cells were present but rare. The large mononuclear cells around the areas of necrosis displayed phagocytic activity and contained intracytoplasmic particles. Elementary bodies or microorganisms were not identified.

Some fibroblastic proliferation and newly formed blood vessels were seen at the periphery of the inflammatory infiltration. The blood vessels displayed slight endothelial swelling but there was no appreciable degree of endothelial proliferation. The lymphatics were prominent and dilated. They contained granular eosinophilic material with some large mononuclear cells and rare lymphocytes. The epidermis at the edge of the ulcer showed some polymorphonuclear leukocytic infiltration, edema, and acanthosis but there was no significant repair.

The Bubonulus

The bubonulus consisted of numerous small and large foci of inflammatory cells throughout the deep layers of the skin of the prepuce. Some foci were well defined, while others merged into the surrounding tissue.

The various stages of the lesion were seen in the smaller foci found

around the larger lesions. In the early stages the adventitia of a small blood vessel in a part of its circumference was infiltrated by large mononuclear cells. This infiltration extended from the adventitia toward the intima and separated the layers of the vessel wall. The endothelium over the involved segment was swollen but showed no appreciable proliferation. The cells showed no disruption and no thrombus formation was encountered (Fig. 6). The perivascular lymphatic space was dilated and contained some lymphocytes and large mononuclear cells in addition to granular eosinophilic material. The wall of the lymphatic next to the blood vessel was incorporated in the inflammatory process and showed some swelling of the endothelium.

This infiltration by large mononuclear cells increased and eventually involved the entire circumference of the vessel. The infiltrating cells extended from the periphery of the vessel wall toward the lumen and produced marked separation of the various layers. This cellular infiltration led to the occlusion of the vessel and formation of a solid granuloma as seen in the primary lesion. Small veins were primarily involved while arterial involvement occurred generally at a later stage.

The small solid granulomata consisted of large mononuclear cells with occasional plasma cells and rare lymphocytes at the periphery (Fig. 7). No intracellular inclusions were found at this stage.

Large granulomatous areas were formed by coalescence of smaller lesions and contained several small blood vessels with compressed lumina. These vessels were inconspicuous and might be overlooked, but the reticulum stain revealed their contours (Figs. 8 and 9). This stain also showed that the stroma throughout the granuloma was intact. The elastic fibers, however, were fragmented or had disappeared. No significant connective tissue proliferation occurred at the periphery of the lesion.

After the blood vessels were obliterated, the center of the granuloma underwent necrosis. The reticulum fibers disappeared in the necrotic area and neutrophilic polymorphonuclear leukocytes appeared. At this stage, the necrotic core consisted of polymorphonuclear leukocytes, some fibrin, and cellular débris (Fig. 10). This was derived from the degeneration of large mononuclear cells and polymorphonuclear leukocytes. The large mononuclear cells around the abscess frequently showed palisading, and a few multinucleated giant cells might also be found. The large mononuclear cells often had ingested cellular débris and nuclear fragments but no other intracellular inclusions were seen. At the periphery of the older foci, plasma cells became more numerous and there were occasional eosinophils. Lymphocytes were not present in appreciable numbers. In this manner small abscesses developed which were often branching or stellate.

The tissue between the granulomata showed edema, some fibrin, and scattered plasma cells and large mononuclear cells as well as occasional lymphocytes. Some proliferation of connective tissue and of new blood vessels was noted around the abscess. A large artery was present throughout the lesion and displayed old thrombosis with almost complete organization and extensive recanalization. This vessel was followed through the various levels of the block. It was noted that a large granulomatous lesion reached the adventitia of the artery and extended into the inner layers of the vessel wall where it had produced thrombosis. The endothelium of the blood vessels and lymphatics was otherwise not remarkable. The lymphatics were not surrounded by a cuff of inflammatory cells but were dilated and contained granular eosinophilic material with a few lymphocytes and large mononuclear cells. The epidermis was not affected by the inflammatory process in the underlying tissues.

The Lesion in the Inguinal Lymph Nodes

The general architecture of the inguinal lymph node was preserved. Stellate abscesses were numerous throughout the cortex but were less frequent in the medulla.

The earliest stage of the lesion consisted of small masses of large mononuclear cells. These masses were found chiefly in the cortex beneath the marginal sinus, but they were present also in the medulla. The mononuclear cells arose outside of the germinal centers of the follicles, and could be distinguished from the cells of the latter by their more distinct contours and acidophilic cytoplasm. There was no necrosis and the reticulum was intact. Some of the solid masses of large mononuclear cells enlarged by merging with adjacent lesions. As a result, the marginal and medullary sinuses were obliterated and the lymphoid follicles disappeared (Fig. 11) but the stroma was preserved. The capillaries, however, were compressed by the proliferation of cells around them. Their lumina were reduced in size and were finally obliterated, but the endothelium showed no appreciable proliferation. Also, there was no fibrosis or increased vascularity. These lesions merged with the uninvolved lymphoid tissue.

The compression and obliteration of the capillaries led to ischemic necrosis in the center of the cell masses and eventually resulted in the formation of abscesses (Fig. 12). The reticulum fibers in the necrotic areas became fragmented and disappeared. Neutrophilic polymorphonuclear leukocytes and some strands of fibrin could then be seen. The leukocytes entered the necrotic zone from the capillaries of the adjacent viable tissue.

The abscesses were identical with those seen in the bubonulus. At

first the necrotic center of the lesion was small and was surrounded by a wide layer of mononuclear cells. Later necrosis spread and the encircling mononuclear cells were reduced to a layer of two or three. Small intracytoplasmic inclusions of bizarre shapes frequently were seen in the large mononuclear cells but no elementary bodies were noted. The reticulum fibers at the edge of the necrosis were slightly condensed. Some new capillaries were formed but the connective tissue did not proliferate to any extent.

The uninvolved portions of the lymph node showed large follicles with prominent and active germinal centers. Young lymphoid elements predominated throughout the cortex and medulla, and the lining cells of the sinuses proliferated. The dilated sinuses contained numerous large mononuclear cells in addition to lymphocytes and some neutrophilic polymorphonuclear leukocytes. Plasma cells were found commonly throughout the parenchyma, sinuses, and capsule of the lymph node. Occasional small collections of large mononuclear cells were encountered around the blood vessels in the capsule. These collections were similar to those described in the primary lesion and in the bubonulus. They had not progressed, however, to the formation of true granulomata with obliteration of the blood vessel. The dilated afferent and efferent lymphatic vessels and the tissues at the hilum of the lymph node were not remarkable.

We have examined also an inguinal lymph node from which the virus ~olated during the recurrence of the disease following a Frei test ͜ ͜ ͜ .t no. 1). This node showed diffuse hyperplasia with active secondary lymphoid follicles. No aggregates of large mononuclear cells or abscesses were seen. There was some increase in small blood vessels and slight fibrosis of the stroma.

Another inguinal lymph node (patient no. 161) was studied which, on aspiration 9 months previously, had yielded the virus of lymphogranuloma venereum. This node showed preservation of the general architecture with a few areas of slight scarring in the cortex and medulla. The small blood vessels and capillaries were increased. The lymphoid follicles were present and often showed active germinal centers. Large mononuclear cells were numerous throughout the node but foci of these cells or abscesses were not found. Some plasma cells and occasional eosinophils were noted. The capsule and the surrounding fat and fibrous tissue showed some fibrosis and slight infiltration by lymphocytes.

COMMENT

This study of lesions, for which the etiologic factor had been proved, shows that the histologic picture of lymphogranuloma venereum is sufficiently distinct to permit diagnosis. The histologic pattern of

lymphogranuloma venereum is identical in the primary lesion, in the bubonulus, and in the lymph nodes of the disease. The early lesion of lymphogranuloma venereum begins with a focal proliferation of large mononuclear cells which form small aggregates. In the lymph nodes these cells appear first in the cortex, while in the primary lesion and in the bubonulus they arise in the adventitia of small blood vessels near the perivascular lymphatic space. The lumina of the lymphatics show no thrombosis or appreciable endothelial changes. In the lymph nodes the large mononuclear cellular infiltration increases and may obliterate the architecture. In the primary lesion and in the bubonulus progression of the lesion causes obliteration of the perivascular lymphatics. The lesion extends from the outer layers of the vessel toward the lumen. The latter is compressed and finally obliterated without associated thrombosis or endothelial proliferation. In the lymph node a similar compression of the capillaries and small blood vessels occurs. As a result, the lesions in the various locations show small solid granulomatous nodules which consist of large mononuclear cells with occasional multinucleated giant cells. Some plasma cells and rare eosinophils are present at the periphery of the nodules.

The obliteration of the blood vessels leads to necrosis in the granulomata. Neutrophilic polymorphonuclear leukocytes enter the necrotic core from the capillaries at the periphery of the lesion. In this manner stellate abscesses are formed which are characteristic of the fully developed lesions.

Intracytoplasmic inclusions ("Gamna bodies") are seen in the large mononuclear cells only after necrosis has occurred. These inclusions are phagocytized cellular débris. We could not identify in sections the elementary bodies of lymphogranuloma venereum which were seen in spreads of mouse brains and yolk sacs infected with the virus.

The solid granulomata as well as the abscesses may coalesce and cause variations in the general pattern of the lesion. In the primary lesion the process involves the uppermost layers of the skin or mucous membrane. The ulcer represents a coalesced mass of granulomata or abscesses which has broken through the surface and has discharged most of its necrotic contents. Secondary infection is commonly superimposed on the ulcer but in our experience does not materially alter the histologic picture. The formation of multiple fistulae in the lymph nodes of lymphogranuloma venereum probably occurs in the same manner as the formation of the ulcer in the primary lesion, but fistulae did not form in our material.

Plasma cells are numerous at the periphery of a fully developed lesion, but few lymphocytes and only occasional eosinophils are present. A similar diffuse inflammatory cellular infiltration is found between the

granulomata and abscesses. In the acute lesions, fibrosis is present but not marked. The lymph nodes taken from a patient recovering from lymphogranuloma venereum likewise showed only slight scarring. Proliferation of the endothelium plays no appreciable part in the formation of the lesion although some new capillaries are formed at the periphery of the abscesses. Thrombosis was encountered only in a single large artery in the bubonulus and was secondary to the extension of a large granulomatous lesion into the vessel wall.

Except for minor differences, our findings of the lesions in the lymph nodes are essentially in agreement with those recorded in the literature. Significant fibrosis was not encountered even in the lymph node excised 9 months after the acute episode. Giant cells were not numerous in our material. The elementary bodies of lymphogranuloma venereum could not be identified in sections, and intracytoplasmic inclusions in the large mononuclear cells were found only after necrosis had occurred. This finding supports the observations of others that these inclusions are phagocytized débris and are not associated with the lymphogranuloma virus.

Necrosis in the lesions of lymphogranuloma venereum does not appear to be primarily a result of the action of the agent on the tissues. The break-down of tissue results from the obliteration of small blood vessels. These are compressed by the infiltration of large mononuclear cells which progress from the adventitia of the vessel toward the lumen. Endothelial proliferation and thrombosis of blood and lymphatic vessels are lacking in lymphogranuloma venereum.

Serial sections were useful in following the sequence of formation of the lesion. This method was particularly valuable in the study of the bubonulus in which the various stages of the lesion were seen in the deep layers of the prepuce uncomplicated by ulceration and secondary infection. Likewise, the bubonulus did not show unrelated previous lesions which commonly disturb the architecture in inguinal lymph nodes.

Most of the histologic descriptions of the primary lesion in lymphogranuloma venereum which we have found in the literature emphasize only the nonspecific character of the lesion in comparison to syphilis. Hansmann,[14] however, has stressed the similarity of the primary lesion to that in the lymph nodes.

The histologic picture of lymphogranuloma venereum differs from that of other known venereal diseases. In the past the lesions of lymphogranuloma venereum in lymph nodes have sometimes been regarded as similar to those of syphilis and have been called lymphogranulomatous gummata.[11] Our experience shows no resemblance be-

tween the lesions of syphilis and lymphogranuloma venereum. Tuberculosis of the lymph nodes might be considered in the differential diagnosis, particularly since tuberculous infection in the Negro produces a wide variety of pictures. The general pattern of the lesion and the absence of acid-fast bacilli differentiate lymphogranuloma venereum from tuberculosis. The marked endothelial proliferation and vascular changes which are striking in chancroid are lacking in lymphogranuloma venereum. The luxuriant granulation tissue of granuloma inguinale with its characteristic Donovan bodies does not resemble the inflammatory infiltration of lymphogranuloma venereum. In our opinion, the diagnosis of lymphogranuloma venereum can be made with reasonable accuracy by histologic examination alone.

One of the lymph nodes in this series is of particular interest. This case (patient no. 1) is reported in detail elsewhere.[21] When first seen, this patient showed clinical and laboratory evidence of acute lymphogranuloma venereum but the attempt to isolate the virus at that time failed. The disease subsided under treatment and the patient appeared clinically well. A Frei test done 6 months later was followed by recurrence of symptoms and enlargement of the previously enlarged inguinal lymph nodes. One of these nodes was excised and the virus isolated from it. On histologic examination the node showed only hyperplasia and some scarring. It is known that in patients who have had lymphogranuloma venereum, the introduction of the antigen in performing the Frei test may cause a generalized reaction after the disease has apparently ceased to be clinically active.[22] The isolation of the lymphogranuloma virus from a lymph node without morphologic evidence of active infection might be interpreted as lending support to the unproved theory that the immunity in virus infections is due to the persistence of the virus in the cells of the host. It is realized, however, that this observation is open to question since the diagnosis of lymphogranuloma venereum was not proved by isolation of the virus when the patient was first seen in this clinic.

Biopsy is not commonly used for the diagnosis of lymphogranuloma venereum. The primary lesion is evanescent and the removal of lymph nodes is generally regarded as inadvisable because of the possible formation of sinus tracts. A number of lymph nodes of lymphogranuloma venereum have been removed in our out-patient department under local anesthesia without serious discomfort to the patient. The incision healed readily and no sinus tracts formed. The bubonulus in the prepuce was excised in the same manner without untoward results. These experiences lead us to believe that biopsy can be useful in the diagnosis of selected cases of lymphogranuloma venereum since more information

can be obtained from the histologic study than from any other single diagnostic procedure.

CONCLUSIONS

The histologic character of lymphogranuloma venereum was studied in twelve specimens. Eight of these were taken from patients in whom the diagnosis of lymphogranuloma venereum was proved by the isolation of the virus. The remaining four patients were considered to have the disease on the basis of their clinical and laboratory findings. These specimens consisted of seven primary lesions, one bubonulus, and four inguinal lymph nodes.

The histologic picture of lymphogranuloma venereum is distinct and is the same in these lesions. Granulomata composed of large mononuclear cells form around small blood vessels which are compressed and obliterated. Thrombosis and endothelial proliferation play no part in the obliteration of the blood vessels which leads to necrosis in the center of the granulomata. Polymorphonuclear leukocytes enter the necrotic core of the granulomata and abscesses are formed. Little fibrosis is seen in either the acute or healed lesions. One of the lymph nodes was removed during recurrence of the disease following a Frei test. This node showed none of the changes of active lymphogranuloma venereum.

The histologic picture of lymphogranuloma venereum is sufficiently distinct to permit a reasonably accurate diagnosis and to differentiate this disease from other venereal infections. For this reason, biopsy appears useful as a diagnostic procedure in selected cases of lymphogranuloma venereum.

REFERENCES

1. Wall, M. J., Heyman, A., and Beeson, P. B. Complement fixation in lymphogranuloma venereum. *Am. J. Syph., Gonor. & Ven. Dis.*, 1947, 31, 289–299.

2. Heyman, A. The clinical and laboratory differentiation between chancroid and lymphogranuloma venereum. *Am. J. Syph., Gonor. & Ven. Dis.*, 1946, 30, 279–286.

3. Ash, J. E., and Spitz, S. Pathology of Tropical Diseases. An Atlas. W. B. Saunders Co., Philadelphia & London, 1945, pp. 12–13 and 18.

4. Boyd, W. A Textbook of Pathology. Lea & Febiger, Philadelphia, 1943, ed. 4, p. 184.

5. Forbus, W. D. Reaction to Injury. The Williams & Wilkins Co., Baltimore, 1943, pp. 496–500.

6. Karsner, H. T. Human Pathology. J. B. Lippincott Co., Philadelphia, Montreal & London, 1942, ed. 6, pp. 243–244.

7. Moore, R. A. A Textbook of Pathology. W. B. Saunders Co., Philadelphia & London, 1944, pp. 444–446.

8. D'Aunoy, R., and von Haam, E. Venereal lymphogranuloma. *Arch. Path.*, 1939, 27, 1032–1082.

9. Greenblatt, R. B. Management of Chancroid; Granuloma Inguinale and Lymphogranuloma Venereum in General Practice. Venereal Disease Information. U. S. Government Printing Office, 1943 (supp. 19), pp 1–43.

10. Koteen, H. Lymphogranuloma venereum. *Medicine*, 1945, 24, 1–69.

11. Stannus, H. S. A Sixth Venereal Disease. Baillière, Tindall & Cox, London, 1933.

12. Pund, E. R., Greenblatt, R. B., and Huie, G. B. The role of the biopsy in diagnosis of venereal diseases; histologic differentiation of venereal granuloma and lymphogranuloma and chancroid. *Am. J. Syph., Gonor. & Ven. Dis.*, 1938, 22, 495–502.

13. Kornblith, B. A. Observations on lymphogranuloma venereum. Clinical pathological study of sixty cases, with observations on the histopathology of the Frei test. *Surg., Gynec. & Obst.*, 1936, 63, 99–109.

14. Hansmann, G. H. Non-tuberculous granulomatous lymphadenitis. *Surg., Gynec. & Obst.*, 1924, 39, 72–82.

15. Bory, L. Le chancre lympho-granulomateux. *Bull. Soc. franç. de dermat. et syph.*, 1921, 21, 451–458.

16. Phylactos, A. Cited by Hansmann.[14]

17. Brandt, R. "Bubonulus" in lymphogranuloma venereum. *Arch. Dermat. & Syph.*, 1940, 42, 811–813.

18. Wall, M. J. Isolation of the virus of lymphogranuloma venereum from twenty-eight patients: Relative value of the use of chick embryos and mice. *J. Immunol.*, 1946, 54, 59–64.

19. Beeson, P. B., Wall, M. J., and Heyman, A. Isolation of virus of lymphogranuloma venereum from blood and spinal fluid of a human being. *Proc. Soc. Exper. Biol. & Med.*, 1946, 62, 306–307.

20. Mallory, F. B. Pathological Technique. W. B. Saunders Co., Philadelphia & London, 1938, pp. 282–283.

21. Heyman, A., Wall, M. J., and Beeson, P. B. The effect of sulfonamide therapy on the persistence of the virus of lymphogranuloma venereum in buboes. *Am. J. Syph., Gonor. & Ven. Dis.*, 1947, 31, 81–86.

22. Hickam, J. B. Cutaneous and articular manifestations in lymphogranuloma venereum. Activation of the disease by the Frei test. *Arch. Dermat. & Syph.*, 1945, 51, 330–336.

[*Illustrations follow*]

DESCRIPTION OF PLATES

PLATE 110

FIG. 1. Primary lesion. The ulcer is surrounded by an inflammatory cellular infiltration. Giemsa's stain. $\times$ 60.

FIG. 2. Primary lesion. A small blood vessel shows beginning infiltration of its walls by large mononuclear cells. The lumen is patent and the endothelium shows no significant proliferation. The single mitotic figure of the endothelium is unusual. Giemsa's stain. $\times$ 710.

FIG. 3. Primary lesion. The infiltration of large mononuclear cells in the wall of the vessel has progressed to the formation of a solid nodule. The lumen of the vessel is completely obliterated by compression but the outline of the vessel is still visible. There is no endothelial proliferation. Giemsa's stain. $\times$ 500.

FIG. 4. Primary lesion. The early stage of a typical abscess showing neutrophilic polymorphonuclear leukocytes in the necrotic center surrounded by large mononuclear cells. To the right a multinucleated cell is seen. Giemsa's stain. $\times$ 420.
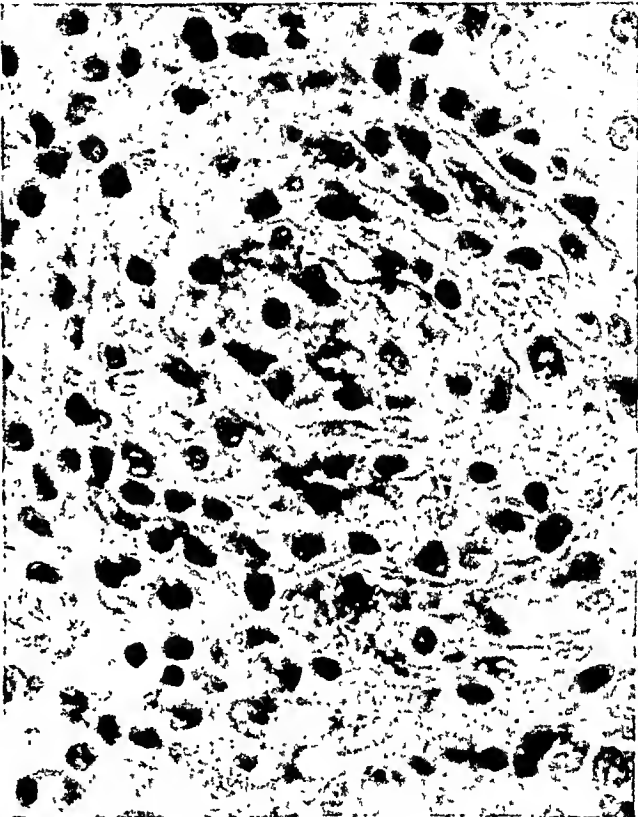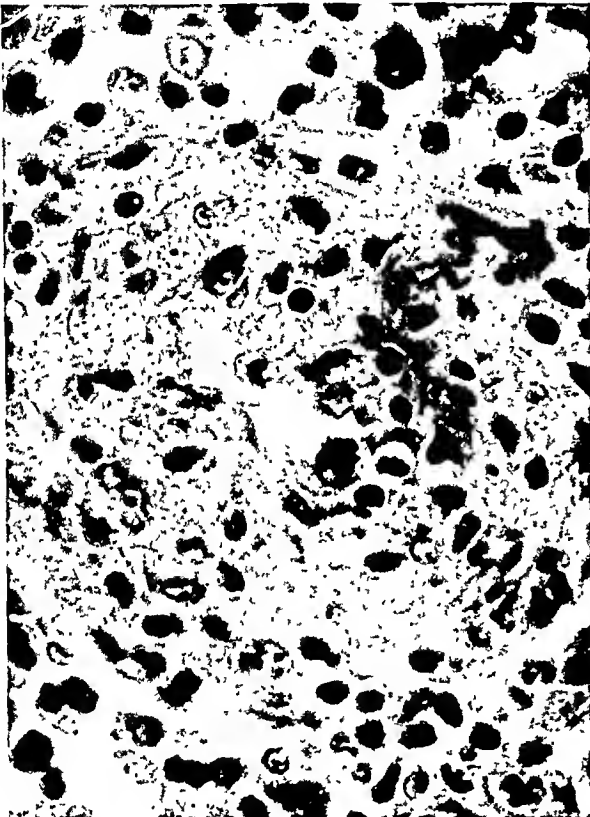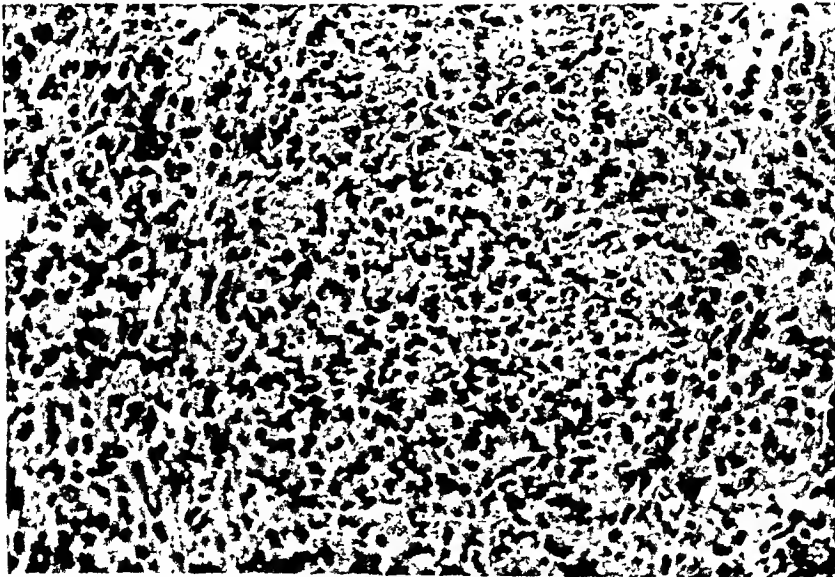
FIG. 5. Primary lesion. The fundus of the ulcer is infiltrated chiefly with large mononuclear cells and is covered with exudate. Below and in the center is a small abscess. Giemsa's stain. $\times$ 170.

FIG. 6. Bubonulus. The lymphatic space around a small vein is dilated and contains large mononuclear cells. These have infiltrated the wall of the vessel. Phloxine and methylene blue stain. $\times$ 345.

FIG. 7. Bubonulus. A large solid granuloma with early necrosis. This lesion has formed by the coalescence of several smaller granulomata. Phloxine and methylene blue stain. $\times$ 150.

FIG. 8. Bubonulus. This lesion consists of multiple solid granulomata which have coalesced. Phloxine and methylene blue stain. $\times$ 140.

PLATE III
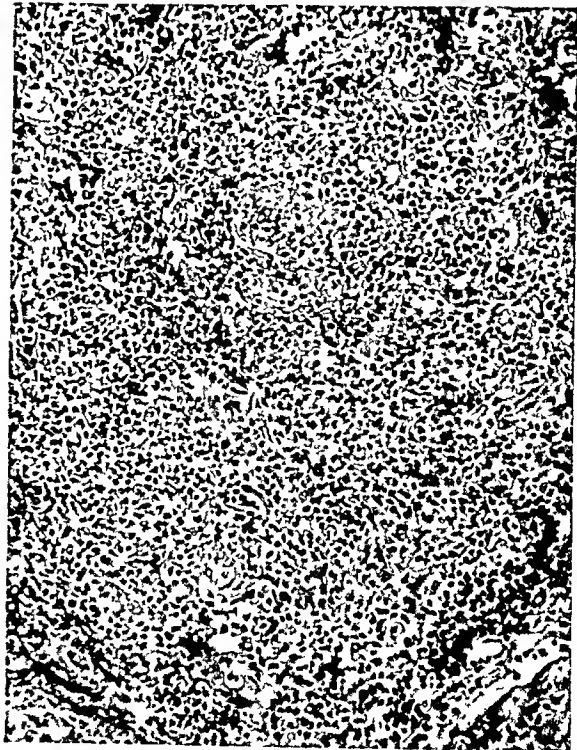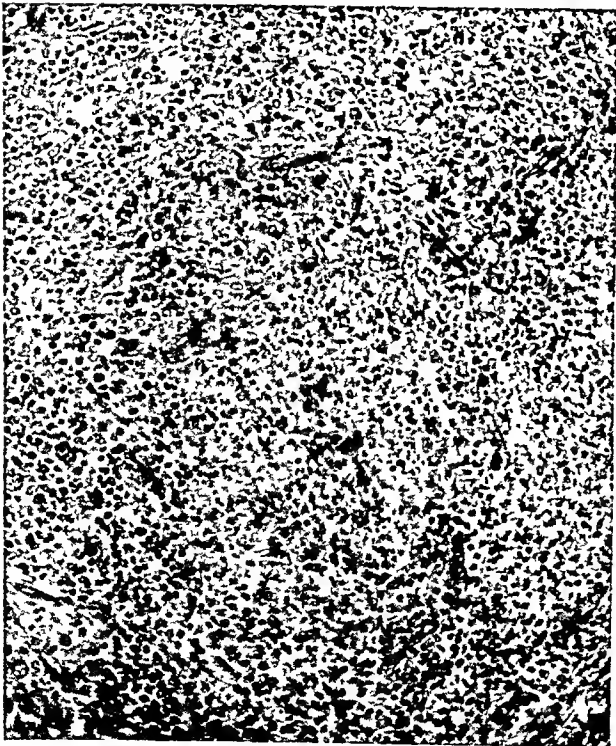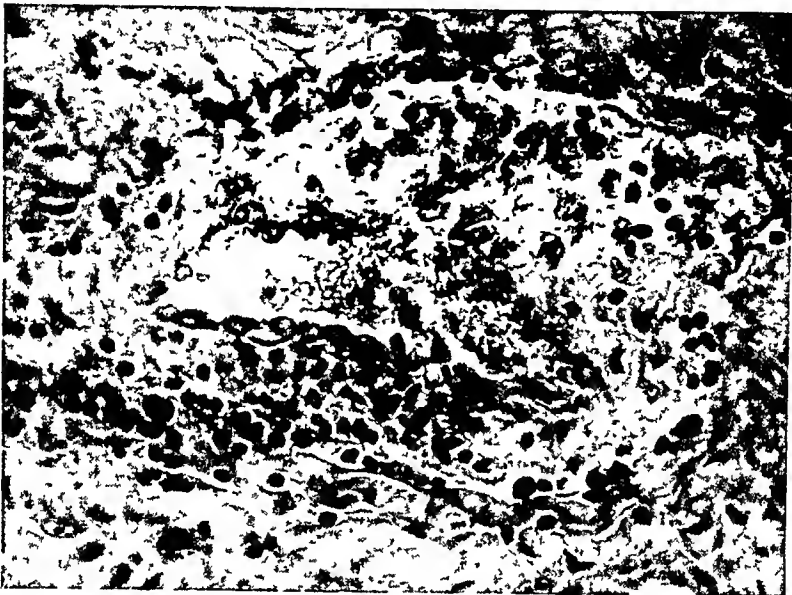
Sheldon and Heyman

Lymphogranuloma Venereum

PLATE 112

FIG. 9. Bubonulus. This is the same field as is shown in Figure 8, but this preparation shows the reticulum and the contours of blood vessels. many of which are masked by the cellular infiltration in Figure 8. Wilder's reticulum stain. × 140.

FIG. 10. Bubonulus. The necrotic core of an abscess with polymorphonuclear leukocytes surrounded by large mononuclear cells. The cavity of the abscess appears branching or stellate. Phloxine and methylene blue stain. × 280.

FIG. 11. Inguinal lymph node. The early stage of the lesion with proliferation of large mononuclear cells and obliteration of the normal architecture. Phloxine and methylene blue stain. × 110.

FIG. 12. Inguinal lymph node, showing a fully developed abscess. Phloxine and methylene blue stain. × 50.
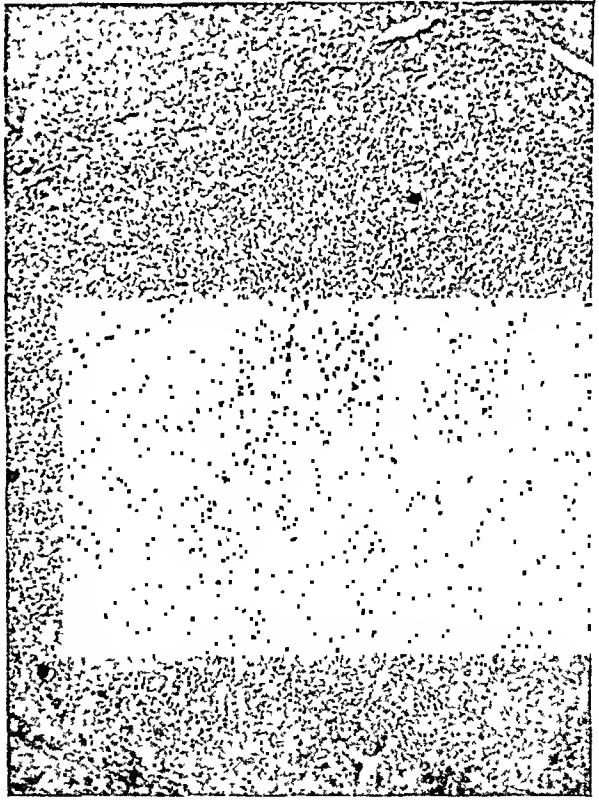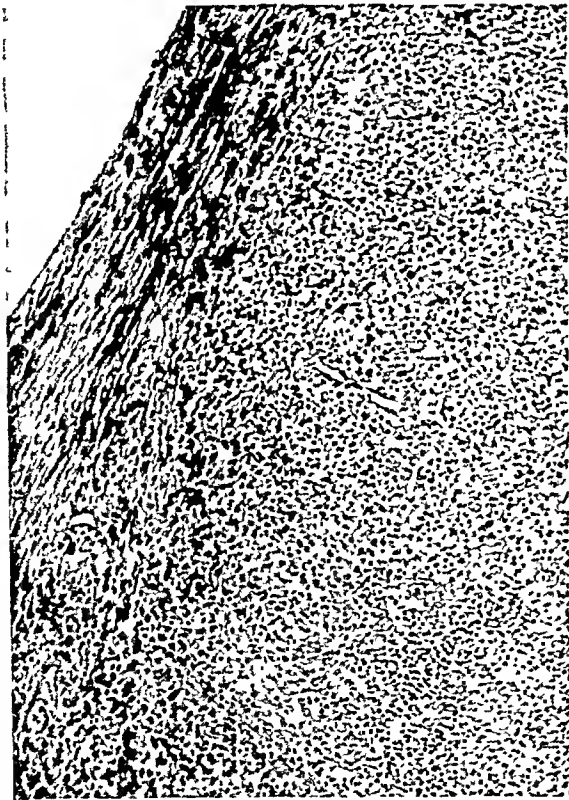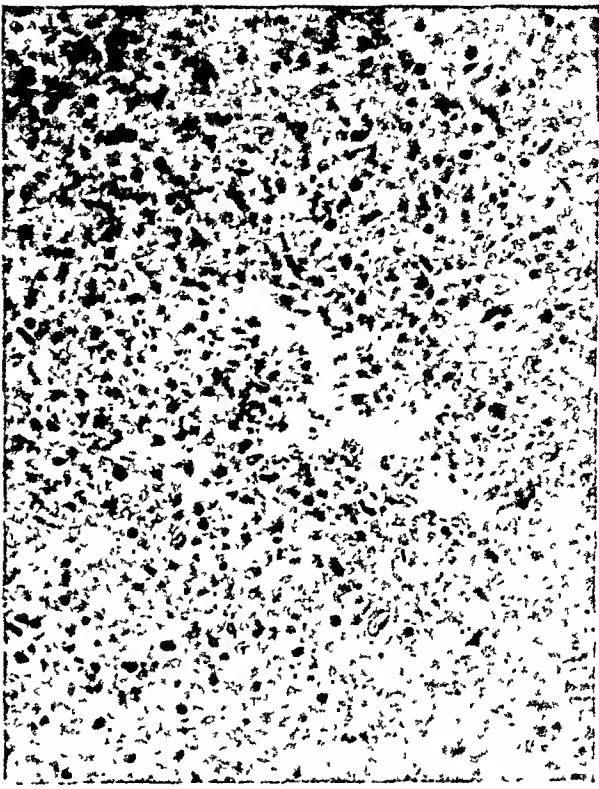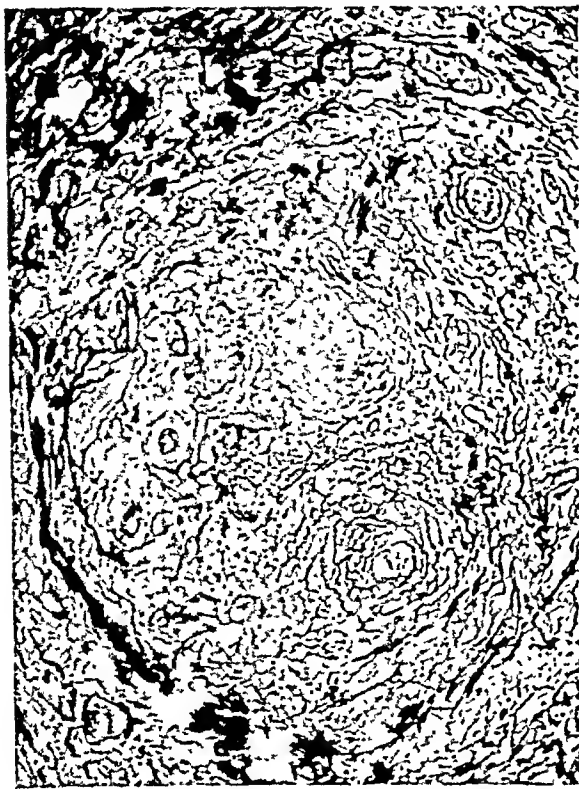
Sheldon and Heyman   Lymphogranuloma Venereum

MUCORMYCOSIS OF THE CENTRAL NERVOUS SYSTEM ASSOCIATED WITH HEMOCHROMATOSIS

REPORT OF A CASE *

PHILIP M. LeCOMPTE, M.D., and WILLIAM A. MEISSNER, M.D.

(*From the U. S. Veterans' Hospital, West Roxbury, Mass., and The Pathological Laboratory of the New England Deaconess Hospital, Boston, Mass.*)

Human infection with fungi of the family Mucoracea is rare. Mucormycosis is not mentioned in the standard American works on pathology, although it is both described and illustrated in one German text.[1] Organisms of this group are referred to as potential pathogens in most works on medical mycology.[2-7] In others they are mentioned as contaminants [8] or "probable pathogens." [9] The literature is reviewed by Gregory, Golden, and Haymaker.[10]

The lungs are the organs most frequently involved, the central nervous system most rarely. The case of Paltauf [11] and the three reported by Gregory, Golden, and Haymaker [10] appear to be the only instances on record of involvement of the central nervous system.

The present case is reported chiefly because of its remarkable resemblance in clinical and pathological details to the three described by Gregory *et al.*[10]

Report of Case

E. A. (R 2104), a white male, 57 years of age, was admitted to the U. S. Veterans' Hospital, West Roxbury, Mass., on September 25, 1944. No adequate history was obtainable. He was semi-comatose, confused, and muttering. The breath had a strong odor of acetone. He did not move his left arm or leg. The right pupil was larger than the left. The right eye was inflamed, swollen, and exuded a purulent discharge. The urine showed 2.5 per cent sugar, a heavy trace of albumin, a few red and white cells, and 3 plus acetone. Blood sugar was 130 mg. per cent; leukocyte count, 12,400, with 84 per cent polymorphonuclear leukocytes, 10 per cent lymphocytes, and 6 per cent monocytes. Hemoglobin was 13 gm. Complement-fixation and precipitation tests for syphilis were negative.

The patient was given 40 units of insulin shortly after admission. The following day he received 100 units with 1000 cc. of 10 per cent glucose. No definite improvement was noted, and he was subsequently given 200 units of insulin with 2000 cc. of 10 per cent glucose. The patient failed to recover consciousness and died on September 27th.

Autopsy (no. 122) was performed by Capt. S. Balkin. The right upper eyelid was found to be markedly swollen and injected. The right pupil was irregular and measured 4 mm. in diameter, while the left one measured 2 mm. About 2000 cc. of clear pale yellow fluid was found in each pleural cavity and 300 to 500 cc. of similar fluid in the peri-

toneal cavity. The heart weighed 450 gm. and appeared negative. The lungs were congested. The liver weighed 2100 gm., was a dusky reddish brown, and showed numerous small, knob-like elevations of its surface. On the cut surface islets of brown tissue were surrounded by depressed gray tissue. The lower end of the esophagus contained dilated veins. The brain showed congestion of the dura and pia, and there were petechial hemorrhages in the pons, medulla, and cerebellum.

Microscopically, the tissues showed the characteristic findings of hemochromatosis, with deposition of yellow pigment granules giving a positive reaction for iron in the liver, spleen, pancreas, adrenals, kidneys, and heart. There was a well marked cirrhosis of the liver.

The brain showed an acute meningo-encephalitis associated with an abundant large, branching fungus (Figs. 1 to 3). The organism consisted apparently of hyphae branching in a nonseptate or coenocytic manner and measuring about 10 to 14 μ in diameter and up to 200 μ in length. The walls of the hyphae were sharply defined and refractile, while the inner portion stained light blue with hematoxylin. The hyphae showed an extraordinary tendency to invade, infiltrate, and replace the walls of blood vessels and to grow within their lumina (Fig. 3). In many places there was an intense reaction of polymorphonuclear leukocytes associated with the presence of the fungus. In other regions, especially where the hyphae lay within the brain substance, as they occasionally did, the reaction was only slight (Fig. 1).

Unfortunately, no culture was obtained from the tissues at autopsy. Sections of the brain were sent to Dr. John E. Gregory, who stated: [12] "Examination of the sections sent to me by Dr. LeCompte reveals invasion by an organism apparently identical with that present in the three cases previously reported."

Slides and tissue were also sent to Dr. C. W. Emmons, Principal Mycologist of the U. S. Public Health Service, who replied: [13] "I concur in Dr. John E. Gregory's opinion that the fungus seen in these sections is probably Mucor because of the size of the hyphae, the infrequency of cross walls and the manner in which the hyphae branch. In the absence of the culture, I am unable to make any more definite identification of the organism."

DISCUSSION

The association of the fungus infection with hemochromatosis in this instance is presumably accidental as far as the disturbed iron metabolism of that disease is concerned. In other respects, however, the case is remarkably similar to those described by Gregory et al.[10] All four cases have exhibited a definite triad, as follows: (1) uncontrolled diabetes;

(2) evidence of orbital infection; (3) meningo-encephalitis associated with the presence of a fungus having the characteristics described above. Another feature common to these cases and mentioned elsewhere in the literature is the extraordinary tendency of the fungus to grow in the walls and lumina of blood vessels. As noted by Gregory *et al.*, much of the necrosis and reaction must be attributed to ischemia produced by the involvement of blood vessels. A similar vascular involvement has been noted in infections with organisms of the Aspergillus group.[6,14]

It seems probable that the fungus gains access to the orbital tissues from the paranasal sinuses and thence invades the brain directly, although such a connection was not definitely demonstrated in the present case. Presumably these cases provide another example of the increased susceptibility of diabetic patients to fungus infections.

SUMMARY

A case of mucormycosis of the central nervous system, apparently the fifth one on record, is described. All four cases reported from this country have had in common the following triad: (1) uncontrolled diabetes, with either coma or mental confusion; (2) evidence of orbital infection; and (3) meningo-encephalitis, with the presence of large, nonseptate, branching hyphae having a peculiar predilection for the walls of blood vessels.

REFERENCES

1. Aschoff, L. Pathologische Anatomie. G. Fischer, Jena, 1936, ed. 8, 1, 213.
2. Castellani, A. Fungi and Fungous Diseases. American Medical Association, Chicago, 1928, pp. 132–133.
3. Conant, N. F., Martin, D. S., Smith, D. T., Baker, R. D., and Callaway, J. L. Manual of Clinical Mycology. W. B. Saunders Co., Philadelphia & London, 1945, p. 199.
4. Dodge, C. W. Medical Mycology. The C. V. Mosby Co., St. Louis, 1935, pp. 109–117.
5. Henrici, A. T. Molds, Yeasts, and Actinomycetes. John Wiley & Sons, Inc., New York, 1930, pp. 66–85.
6. Brumpt, E. Précis de parasitologie. Masson et Cie., Paris, 1936, ed. 5, 2, 1991; 2040–2050.
7. Kolle, W., and Wassermann, A. v. Handbuch der pathogenen Mikroorganismen. G. Fischer, Jena, and Urban & Schwarzenberg, Berlin & Wien, 1928, 5, Pt. 1, 159–163; 177.
8. Lewis, G. M., and Hopper, M. E. An Introduction to Medical Mycology. The Year Book Publishers, Chicago, 1939, p. 305.
9. Swartz, J. H. Elements of Medical Mycology. Grune & Stratton, New York, 1943, pp. 157–158.
10. Gregory, J. E., Golden, A., and Haymaker, W. Mucormycosis of the central nervous system. A report of three cases. *Bull. Johns Hopkins Hosp.*, 1943, 73, 405–419.

11. Paltauf, A. Mycosis mucorina. *Virchows Arch. f. path. Anat.,* 1885, 102, 543–564.

12. Gregory, J. E. Personal communication.

13. Emmons, C. W. Personal communication.

14. Lang, F. J., and Grubauer, F. Über Mucor- und Aspergillusmykose der Lunge. *Virchows Arch. f. path. Anat.,* 1923, 245, 480–512.

DESCRIPTION OF PLATE

PLATE 113

FIG. 1. A nonseptate branching hypha in the brain. In this instance the reaction of the tissue is relatively slight. Hematoxylin and eosin stain. × 500.

FIG. 2. Hyphae in the brain tissue, accompanied by a marked polymorphonuclear leukocytic reaction. Hematoxylin and eosin stain. × 500.

FIG. 3. Small meningeal vessel in a cerebral sulcus. The fungus has almost replaced the wall of the vessel and is growing in the lumen. Hematoxylin and eosin stain. × 500.
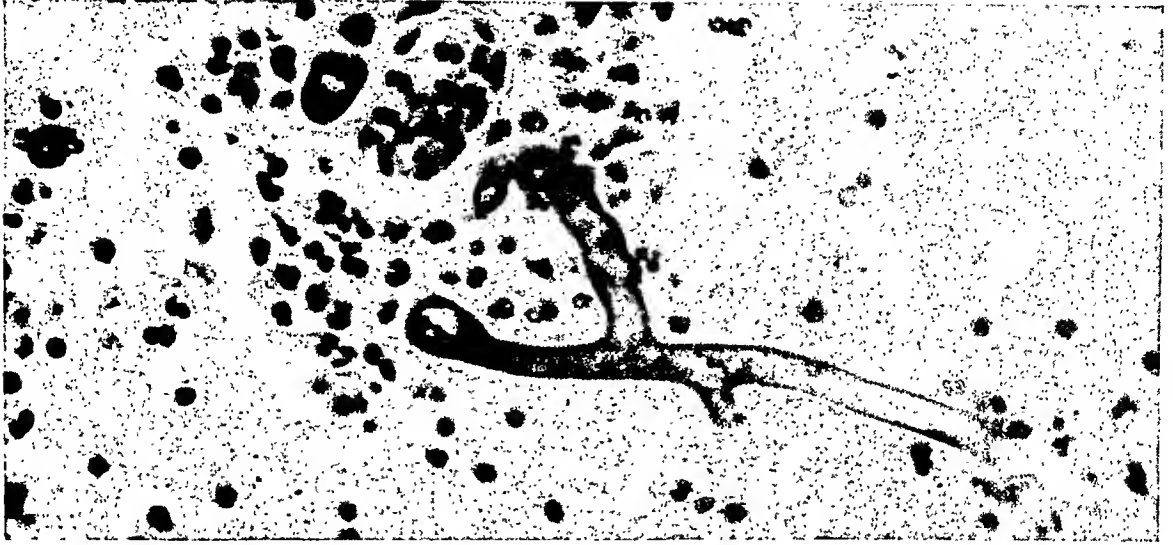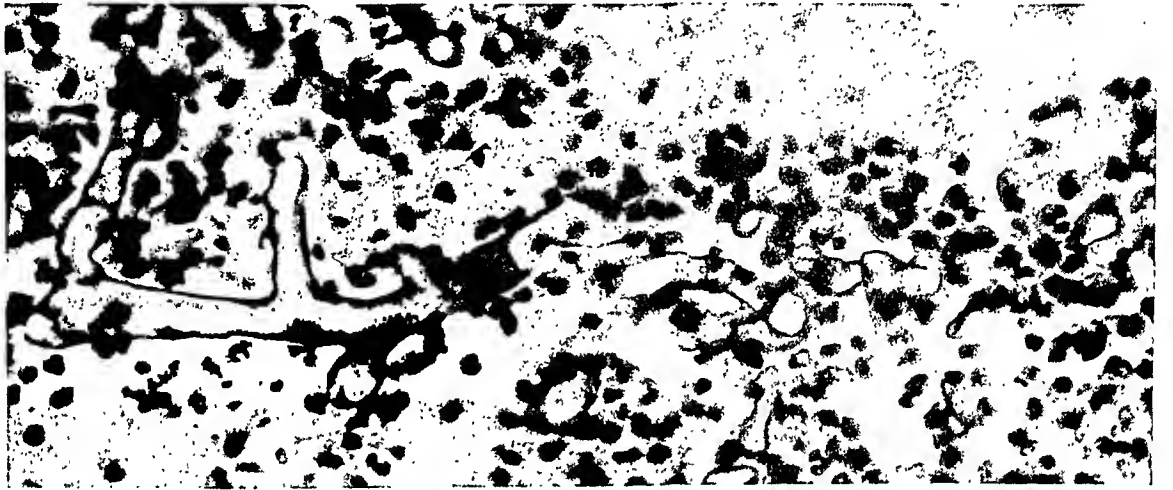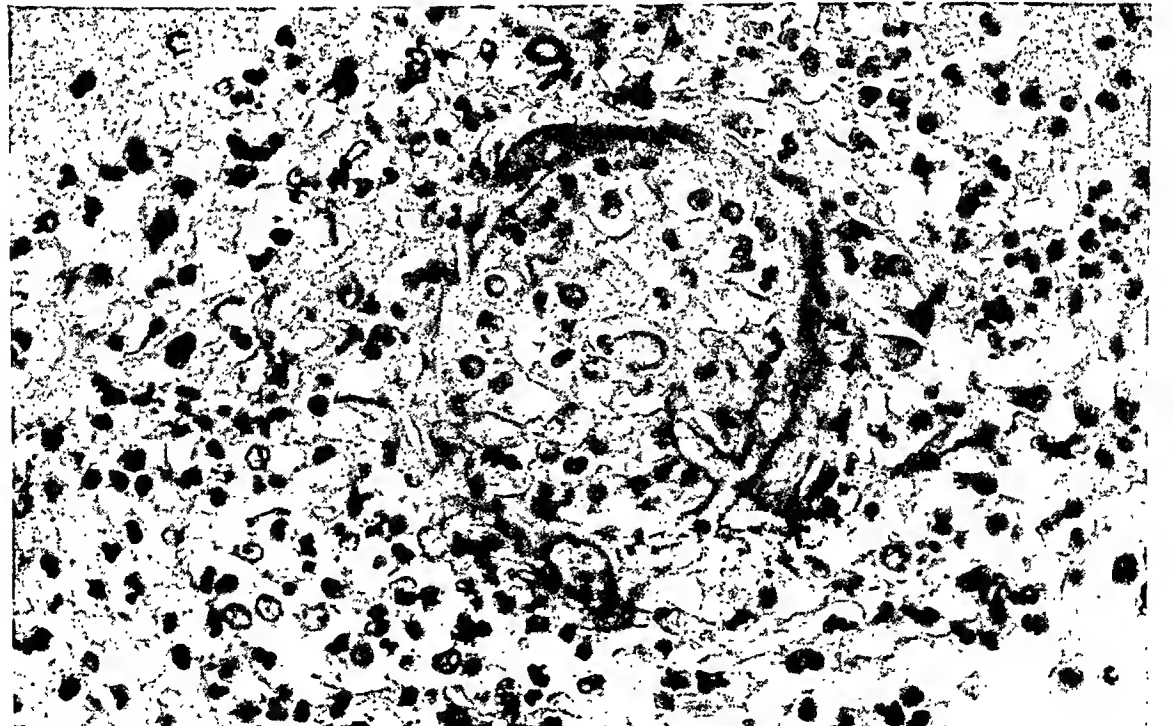
LeCompte and Meissner

Mucormycosis with Hemochromatosis

PULMONARY CHANGES FOLLOWING EXPOSURE TO PHOSGENE *

STANLEY H. DURLACHER, M.D.,† and HENRY BUNTING, M.D.

(From the Department of Pathology, Yale University School of Medicine, New Haven, Conn.)

The pathologic changes in the respiratory tract following exposure to phosgene have been described by Winternitz[1] and Vedder.[2] No mention was made of pulmonary consolidation such as is described in this report. This was encountered during studies of the therapeutic effect of 60 per cent oxygen upon dogs surviving exposure to phosgene. It was responsible for the late deaths from anoxia occurring after recovery from the earlier anoxia associated with pulmonary edema.

METHODS

Dogs were observed for 3 to 4 days prior to gassing and only such animals as appeared healthy were utilized. Exposure to phosgene was maintained for 30 minutes in a dynamic chamber at an average concentration of 0.29 mg. per L. After exposure animals were treated according to the following procedures:

A. No treatment.

B. 10 cc. per kg. of blood removed after exposure.

E. Small amounts of plasma administered intravenously as indicated by fall in blood pressure or tachycardia.

F. Maintained in an atmosphere of 60 per cent oxygen when the arterial oxygen saturation was below 80 per cent. Plasma as in group E.

G. Oxygen treatment as in group F. No plasma.

H. Maintained in 60 per cent oxygen.

The dogs used in this study have been divided according to survival after exposure, into four groups, as defined subsequently.

At autopsy an incision was made in the neck and a clamp placed firmly about the trachea to prevent escape of fluid from the respiratory

tree. The ratio of lung to body weight was determined; a ratio of 0.015 was found to be the upper limit of normal. Tissue for microscopic section was fixed in 10 per cent neutral formalin. Paraffin sections stained with hematoxylin and eosin, Masson's stain for connective tissue, Weigert's stain for elastic fibers, and phosphotungstic acid-hematoxylin stain for fibrin were used.

RESULTS

Summary of Alterations in Oxygenation of Blood Following Exposure to Phosgene. Immediately after exposure to phosgene, dogs showed a transient fall in the oxygen saturation of the arterial blood, due presumably to bronchiolar constriction. Several hours later there was a progressive, more profound anoxemia caused by pulmonary edema; death often occurred during the first 48 hours after exposure. The oxygen saturation of surviving animals gradually returned to normal. Administration of 60 per cent oxygen to dogs during the stage of pulmonary edema caused a temporary rise in arterial oxygen saturation and reduced the number of deaths. However, a later phase of anoxemia 2½ to 4 days after exposure was observed in a few untreated animals that had survived the period of pulmonary edema. It was the rule in comparable animals that had received oxygen. This late anoxemia accounted for the fact that the mortality rate after oxygen was not significantly lower than for untreated animals.[3]

Anatomical Changes Following Phosgene. The essential pulmonary findings in the dogs comprising this report are outlined in Table I.

Group I. Seven Dogs Surviving Not More Than 3 Days after Gassing

Gross Findings. The lungs were voluminous and heavy. Below the smooth, moist, transparent pleural surface there were alternate soft, pale pink, emphysematous areas and dark red, depressed, firm, non-crepitant, edematous areas which pitted on pressure. When sectioned, considerable quantities of pale yellow, occasionally blood-tinged fluid and froth poured from the tissue and bronchi. The loose peribronchiolar tissue and the subpleural and interlobular lymphatics were distended by fluid. The major bronchi were filled with frothy fluid; their mucosal surfaces were smooth and pale.

Microscopic Findings. The serosal surface of the pleura was free of exudate. Patches of enlarged, air-containing, thin-walled alveoli were found interposed between zones of smaller alveoli filled with pink-staining, protein-rich fluid and strands of fibrin; rare polymorphonuclear leukocytes, small numbers of red blood cells, and occasional mononuclear cells were present in the exudate. The capil-

TABLE I

Summary of Anatomical Findings after Exposure to Phosgene

Dog no.	Time post-gassing	Lung/body ratio	Gross findings Edema	Gross findings Emphysema	Gross findings Atelectasis	Gross findings Consolidation	Alveolar exudate Edema	Alveolar exudate Fibrin	Alveolar exudate Red blood cells	Alveolar exudate Polymor-phonuclear cells	Alveolar exudate Histiocytes	Alveolar exudate Organization	Alveolar walls Congestion	Alveolar walls Cellular exudate	Alveolar walls Scars	Bronchioles Mucosal necrosis	Bronchioles Cellular exudate	Bronchioles Organization	Bronchioles Scars
Group I																			
1A*	16 hrs.	0.029	+++	+++	++		+++	++	+	+			++			++			
2F	35 hrs.	0.059	++++	++	+		++++	+++	+	+			++			+++			
3F	40 hrs.	0.042	+++	+	+		+++	+++	+	+			+			+++			
4H	42 hrs.	0.049	++	+++	+++		++	++	+	+			+			++			
5B	42 hrs.	0.034	++++	++	+++		++++	++	++	++++	++		+	+		+	++	+	
6G	60 hrs.	0.036	++	+	+		++	++	+	++	++		+	+		++	+++	+	
7H	72 hrs.	0.029																	
Group II																			
8G	4 days	0.027	+	++		++	+	++	++	+	++	+	++			++	+	++	
9F	4 days	0.048	++	+	+	+	+	+	+		++		+			++	+	+	
10H	4 days	0.019	+	++	++	+++	+	+			+	++	+++					+++	
11G	4½ days	0.021			+	++	++	+	++		+	+++	+++				+	+++	
12H	4½ days	0.035	+	+	+	+	++	+	++		+++	+++	+++			++	+	++	
13G	4½ days	0.030	++		+	++	+++	+	+		+	+++	+++				+	++	
14H	4½ days	0.016	+	+	+	+	++	+		+	++	+++	+++				+	+++	
15E	4½ days	0.016	++				+		+		+	+++	+++					++	
16H	5 days	0.014	+	++	+	+++	+	++	++		++++	+++	+++			+	++	+++	
17F	5 days	0.026				+++		+	+		+++	+++	+++				+	++	
18H	5½ days	0.034	+	+	+	+	+				+	++	+++				++	+	
19A	6 days	0.026				+++	+	++	+	+	+	++	++		+	+	+++	+++	
20H	7 days	0.015	+	+	+	+++			+	+++	+	+++	+++			+	++++	++	
21H	7 days	0.025		++		+				+	+	+	+++				+	+	
22H	8 days	0.032	+	+++	++	+++	+	+	+		+	+++	+++		+			+++	
23H	8 days	0.033		+	+	+++		+	+		++	+	+++					++	
24H	9 days	0.014		+							+	++	+++		+	+		+++	++
25E	9 days	0.023			++	+++	+	+			+	++	++++		+	++		++	+++
Group III																			
26G	27 days	0.016		++	++										+++	+			
27G	59 days	0.013			++										++	++			
Group IV																			
28		0.010																	
29		0.015													+				
30		0.015		+															
31		0.017		+															

* The letters indicate the procedure employed following gassing.

laries of the alveolar walls were congested but no thrombi were noted. Necrosis of alveolar structures was not observed, but there was necrosis of the mucosa of small bronchioles with plugs of desquamated epithelial cells, fibrin, and necrotic débris within the lumina. Metaplastic changes of the regenerating bronchiolar epithelium were found in those dogs that survived longest in this group. The major bronchi and blood vessels showed no changes. In dog 6 purulent bronchitis and focal pneumonia were found.

Group II. Eighteen Dogs Surviving 4 to 9 Days after Gassing Irrespective of Treatment

Gross Findings. The pleural surface was transparent and free of exudate. The lungs showed emphysema and edema but these were not so extensive as in the early post-gassing period. In the majority of instances there was consolidation of one or more lobes with firm pale parenchyma of rubbery consistency (Fig. 1). The cut surface of these lobes was dry, gray, and granular, and bulged above the pleura (Fig. 2). The bronchi gaped widely and contained mucoid material. In the non-consolidated regions small, raised, gray, glistening, tubercle-like lesions were observed, apparently surrounding small bronchioles. No changes were noted in the major blood vessels.

Microscopic Findings. There was no exudate on the serosa of the pleura. During the fourth and fifth days after gassing, small amounts of fluid, fibrin, red blood cells, and remnants of polymorphonuclear leukocytes persisted in the alveoli. In most places this material had coagulated into an amorphous, deep-red-staining mass. The alveolar lining cells were swollen and obscured the alveolar walls. Later, the alveolar walls were infiltrated and the alveoli filled with large macrophages having round or oval, vesicular nuclei with the chromatin distributed about the periphery and a prominent, centrally placed nucleolus (Fig. 3). The involved parenchyma was diffusely consolidated and the architecture ill defined. At this stage, the bronchioles were the only structures in which organization could be seen (Fig. 4). In those animals surviving longer, well formed fibroblasts were present within the alveoli (Fig. 5), and collagen was detectable by the Masson technic. Where alveolar walls could be clearly identified by elastic tissue stain, it could be observed that organization did not arise from intact alveolar walls, but appeared to extend into the alveoli from points of attachment in the bronchioles. At this stage the involvement of the lobes was not diffuse. Organization of alveolar exudate was confined to focal areas with a peribronchiolar distribution; the intervening alveoli were air-containing and their walls thick and cellular. Plugs of tissue of organization, covered by proliferating islands of cuboidal or squamous

epithelium, entirely or partly occluded the bronchioles in all animals except dog 20 (Fig. 6). This animal had a suppurative bronchiolitis and focal pneumonia unaccompanied by the proliferative processes described above.

Group III. Two Dogs That Had Shown Anoxia 4 to 9 Days after Exposure but Had Recovered

Gross Findings. There were no pleural adhesions. Patchy emphysema and small foci of atelectasis were found beneath the smooth, glistening pleura. The pulmonary parenchyma was crepitant, but an increased resistance with a rubbery consistency, particularly of the lower lobes, was noted. When sectioned, the parenchyma presented a dry, spongy, pink surface. In the subpleural region on the posterior surfaces of the lower lobes in dogs 26 and 27 there was a thin band of firm, white, noncrepitant tissue. No changes were observed in the major bronchi or blood vessels.

Microscopic Findings. The alveoli were free of exudate. Thickening of the alveolar walls by collagen and mature fibrocytes was present in a few focal zones and also diffusely in other areas. Many bronchioles were partially or completely obstructed by fibrous connective tissue plugs, the lumina frequently showing "recanalization" around the plug with re-epithelialization of the air passages (Fig. 7). In dogs 26 and 27 there was atelectasis of the subpleural parenchyma in the posterior portions of the lower lobes where scarring of the bronchioles was so marked as to result in complete obstruction (Fig. 8). A few small adventitial hemorrhages were present about the larger pulmonary vasa.

Group IV. Four Dogs Not Exposed to Phosgene but Maintained Continuously in an Atmosphere of 60 Per Cent Oxygen for 7 Days before They Were Examined

Gross Findings. There were no significant gross changes. A few small emphysematous blebs were present in the anterior margins of the upper lobes.

Microscopic Findings. The alveolar walls were intact and the lumina free of exudate. The bronchial tree was lined by well preserved mucosa and showed no change. A few small foci of emphysema and atelectasis were observed.

PROTOCOLS OF REPRESENTATIVE ANIMALS FROM GROUPS II AND III
Dog 17, Group II

Mongrel, male, weight 15.4 kg. Maximum hemoconcentration of 46 per cent was attained 17 hours post-gassing, at which time an arterial oxygen saturation of less than 80 per cent was first observed. The ani-

mal was placed in an atmosphere of 60 per cent oxygen 3 hours later, when the oxygen saturation of the arterial blood was 70 per cent. A minimum arterial oxygen saturation of 68 per cent was observed 28 hours post-gassing, while the animal was breathing 60 per cent oxygen. Plasma in the amount of 2.6 cc. per kg. was administered in two doses 15 hours post-gassing. Oxygen therapy was continued for 54 hours; at this time the arterial oxygen had returned to 88 per cent saturation. A fall in saturation to 57 per cent was observed when the animal was removed from the oxygen chamber, 3 days post-gassing. There was subsequent recovery in room air to 85 per cent saturation 5 days post-gassing.

Gross Findings. The lungs weighed 400 gm. There was emphysema and congestion of the upper lobes bilaterally. A few small foci of depressed, noncrepitant pulmonary parenchyma of moist consistency were also noted. The middle and lower lobes were completely consolidated, noncrepitant, and rubbery. On cut section their parenchyma bulged above the pleura and was granular and dry.

Microscopic Findings. The pleura was thin and the parenchyma below it completely without air-containing tissue. The structure of the alveolar walls was not recognizable, and the tissue within the alveoli consisted of a solid mass of young proliferating fibroblasts and large mononuclear phagocytes. Many of these mononuclear cells showed mitotic figures. The terminal bronchioles were filled with plugs of proliferating epithelium of squamous and cuboidal type, supported and nourished by a stroma of young capillaries and fibroblasts. The mucosa of the smaller bronchi showed extensive metaplastic change. The entire bronchus in places was lined by a layer of squamous epithelium three or four cells deep. The larger bronchi, on the other hand, showed no mucosal changes. The submucosa was free of exudate and the lumina contained a few polymorphonuclear leukocytes and pink amorphous substrate. The walls of the blood vessels showed no changes. There was no evidence of thrombosis or ring hemorrhage.

Dog 23, Group II

Mongrel, female, weight 11.8 kg. The animal was placed in a chamber containing 60 per cent oxygen 25 minutes post-gassing and a maximum hemoconcentration of 30 per cent was attained in 19 hours. A minimum arterial oxygen saturation of 49 per cent was observed 30 hours after exposure to phosgene with subsequent recovery to 70 per cent 55 hours later. Four days after gassing, the arterial oxygen saturation was 64 per cent and there was a further progressive decline until death 8½ days post-gassing. The arterial oxygen saturation was 35

per cent 3 hours before death. The animal had remained in 60 per cent oxygen uninterruptedly until death.

Gross Findings. The lungs weighed 390 gm. The pleural surfaces were smooth and glistening. There were blebs of emphysema in the upper lobes, particularly along the anterior margins. Severe emphysema was noted in the post-cardiac and middle lobes. There was dilatation of the subpleural lymphatics, particularly over the lower lobes. Both lower lobes were firm and noncrepitant. They maintained their shape, and on cut section firm, rubbery tissue bulged above the pleura. The parenchyma was pale, gray, granular, and opaque. The bronchi and bronchioles gaped widely and contained a small amount of viscid material. There was no pus within the bronchi and no fluid or exudate could be scraped from the surface of the lower lobes. The upper lobes were moist on cut section and showed alternate zones of congestion and emphysema.

Microscopic Findings. Throughout all sections the alveoli were filled with large cells containing vesicular oval nuclei and abundant, blue-staining cytoplasm which gave off fibrillary processes. When stained by the Masson technic, these processes took up the green dye. The alveolar walls were preserved throughout all lobes and the young tissue of organization within the alveoli did not appear to be attached to the alveolar walls. The bronchioles showed plugs of young tissue which filled the lumina. In the upper lobes the organization within the alveoli was not as diffuse and alternate zones of emphysema, atelectasis, and hemorrhage were noted in the parenchyma.

Dog 25, Group II

Mongrel, female, weight 15.7 kg. Maximum hemoconcentration of 37 per cent above immediate post-gassing value was attained 14 hours after exposure to phosgene. At this time an arterial oxygen saturation of less than 80 per cent was first observed; progressive fall continued until 48 hours post-gassing, when a minimum saturation of 49 per cent was attained. Two courses of plasma totaling 5.0 cc. per kg. were administered 14 and 20 hours post-gassing, respectively. The arterial oxygen saturation remained below 80 per cent for a total of 7 days, returning to 84 per cent on the eighth day. It was 83 per cent the following day, when the animal was sacrificed with sodium pentobarbital intravenously.

Gross Findings. The lungs weighed 355 gm. They were heavy and of a rubbery consistency throughout. In the upper lobes there was some crepitation but the lower lobes appeared to be entirely solid. Small zones of marginal emphysema were apparent in the upper lobe.

On cut section the lower lobes were airless and showed an unusually pale, granular, firm, dry surface. The upper lobes were pale and had an increased resiliency.

Microscopic Findings. The alveolar spaces were filled with fibroblasts and there was well formed collagen within the alveolar spaces. The alveolar walls were preserved, as could be recognized in sections stained for elastic fibers. There was no other cellular exudate in any of the alveoli and there was no evidence of edema or hemorrhage in either upper or lower lobes. In the upper lobes the distribution of connective tissue in the alveoli was patchy, whereas in the lower lobes it was diffuse and confluent. The smaller bronchioles were plugged with masses of tissue of organization. The large bronchi and blood vessels were free of change.

Dog 27, Group III

Mongrel, male, weight 12.5 kg. Maximum hemoconcentration of 40 per cent was attained 12 hours post-gassing, at which time an arterial oxygen saturation of less than 80 per cent was first observed (77 per cent). The animal was placed in an atmosphere of 60 per cent oxygen 1 hour later. A minimum arterial oxygen saturation of 63 per cent was observed 40 hours post-gassing, while the animal was breathing 60 per cent oxygen. Four days post-gassing the oxygen saturation had risen to 82 per cent, but thereafter slowly fell to another point of minimum saturation (69 per cent) 8 days post-gassing. Gradual recovery was resumed and the arterial oxygen saturation was 92 per cent just before removal of the animal from the oxygen chamber 17 days post-gassing. It was sacrificed with sodium pentobarbital intravenously 50 days post-gassing; the arterial oxygen saturation was 91 per cent at that time. Momentary removal from the oxygen chamber at 3 and 7 days post-gassing was followed by prompt collapse and respiratory arrest, with as rapid recovery when the animal was returned to an atmosphere of 60 per cent oxygen and given artificial respiration. The body weight fell to 8.9 kg. 19 days post-gassing and was 9.4 kg. at the time of death, although the dog appeared healthy otherwise and was eating well.

Gross Findings. The pleura was thin and transparent except over the posterior surfaces of both lower lobes, where a zone of thick, white, firm, opaque tissue, 0.5 cm. in thickness, was present in the subpleural region. The remaining lung was pink and crepitant but had an increased resiliency throughout. There were a few small, depressed, dark red, noncrepitant, atelectatic zones in the post-cardiac and right lower lobe. The major bronchi and blood vessels showed no changes.

Microscopic Findings. The pleura was thin, and below it, in the post-cardiac lobe and in both lower lobes, were thin zones of atelectatic pul-

monary parenchyma. These zones were associated with small bronchioles which were completely obstructed by dense fibrous connective tissue plugs. In some areas there was marked emphysema. Elsewhere the alveolar walls were well preserved but were thickened by collagen fibers and numerous fibrocytes. The bronchioles throughout the sections showed complete or partial obstruction of their lumina by plugs of scar tissue, the surfaces of which were covered by columnar epithelium. The major bronchi were well preserved, as were the blood vessels. The blood vessels showed some edema of the adventitia, but no other change.

DISCUSSION

While proliferation of fibroblasts with organization of exudate in and about small bronchioles has been reported in dogs following exposure to phosgene, the extension of this process to involve an entire pulmonary lobe has not hitherto been encountered.[1] The development of lobar consolidation is dependent upon two factors: severe pulmonary injury due to phosgene, and a survival period adequate for the development of the lesion.

The dogs of group II with severe pulmonary injury due to phosgene survived the phase of pulmonary edema because of oxygen therapy. They subsequently developed anoxia, not relieved by oxygen, that resulted from extensive pulmonary consolidation. That this organization was not a consequence of oxygen therapy is indicated by the occurrence of similar lesions in 3 animals not given oxygen after exposure, and by the absence of pulmonary changes in control animals exposed to oxygen alone for a comparable period.

The paucity of polymorphonuclear leukocytes in the exudate of the majority of instances indicates that the consolidation was not the result of an infectious process. This is borne out by the absence of organisms in aerobic cultures of the lungs of 3 animals. Dog 20 had a purulent pneumonia presumably of bacterial origin and differed from the others of this group, in which histiocytic and fibroblastic proliferation predominated.

Microscopic study of lungs stained with Weigert's elastic tissue stain indicated that destruction of the alveolar walls had not occurred in the consolidated lobes. The granulation tissue within the alveoli did not appear to originate from the alveolar walls but rather to extend into the adjacent alveoli from points of attachment within a terminal bronchiole. These observations explain why so few parenchymal scars were observed in the 2 dogs of group III which survived an episode of anoxia during the period of 2½ to 4½ days after gassing. The discrepancy between the amount of exudate at the stage when organization had

begun and the amount of ultimate scar tissue indicates that more of the exudate had resolved than would have been anticipated. This may be explained by the fact that only minor alveolar damage is caused by phosgene and by the absence of persistent chemical or bacterial irritating agents within the alveoli. The only large scars in the lungs of these animals were found in the bronchiolar lumina. It is noteworthy that in both of these dogs there were a few areas where the alveolar walls were slightly thickened by mature collagen fibers. Such interstitial scarring might be interpreted as reflecting earlier damage to the alveolar walls by phosgene.

SUMMARY AND CONCLUSIONS

1. Consolidation of one or more lobes of the lung has been found in dogs 4 to 9 days after exposure to phosgene (0.29 mg. per L. for 30 minutes). In addition to "obliterative bronchiolitis," the involved lobes showed a diffuse mononuclear exudate within the alveoli and foci of thickening and organization of the alveolar walls composed of large mononuclear cells and young fibroblasts.

2. Pulmonary organization occurred as the initial edema subsided. This resulted in severe late anoxemia and caused high mortality in spite of oxygen therapy during the period.

3. Two dogs that had survived the stage of pulmonary organization, as indicated by "clinical" observation, showed only focal scars in the pulmonary parenchyma and bronchioles 27 and 59 days after gassing.

REFERENCES

1. Winternitz, M. C. Collected Studies on the Pathology of War Gas Poisoning. Yale University Press, New Haven, 1920.
2. Vedder, E. B. The Medical Aspects of Chemical Warfare. Williams & Wilkins Co., Baltimore, 1925.
3. Ordway, N. K., Harrison, H. E., Bunting, H., and Albrink, W. S. Unpublished data.

DESCRIPTION OF PLATES

PLATE 114

FIG. 1. Dog 23 (8 days). Consolidation of all lobes with subpleural emphysema.

FIG. 2. Dog 23 (8 days). Cut section of right lower lobe, showing the solid character of the parenchyma.

Plate 114

Durlacher and Bunting

Pulmonary Changes Following Phosgene
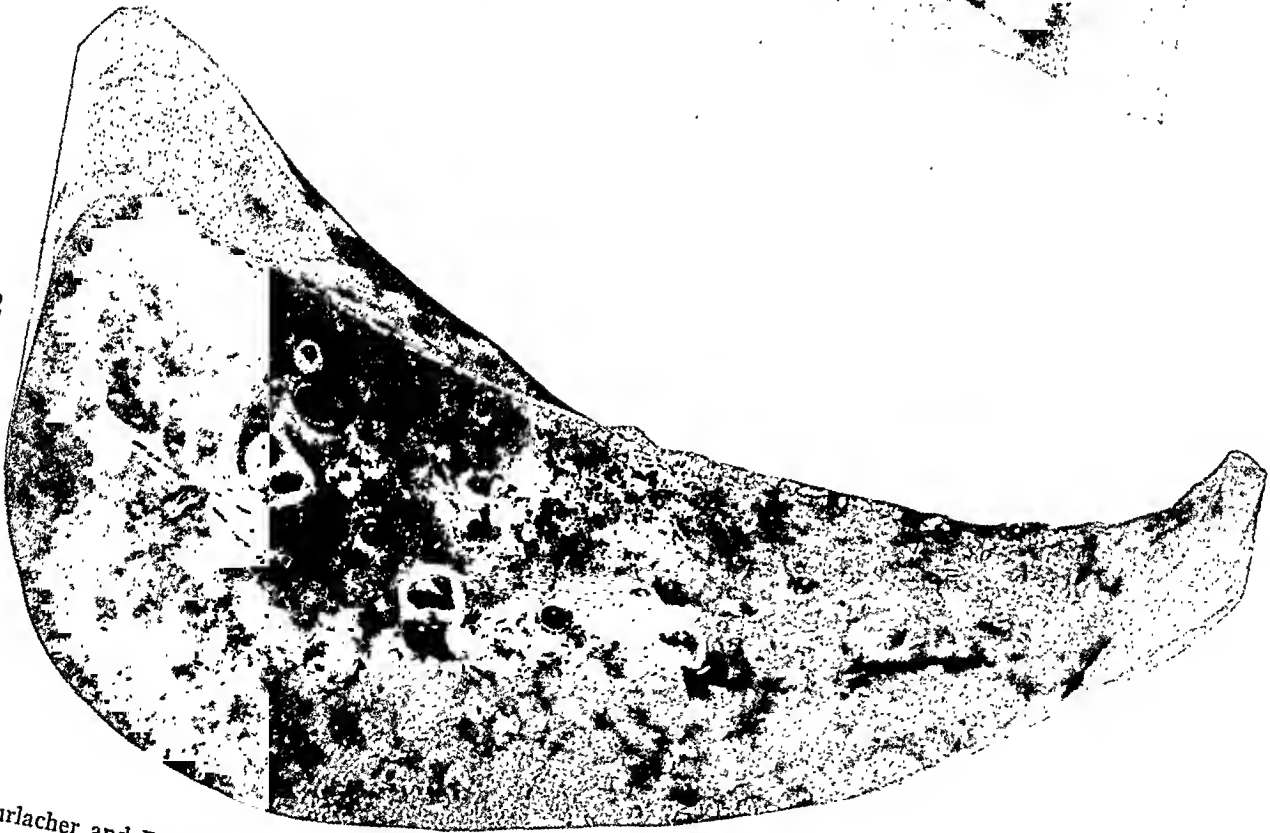
FIG. 3. Dog 23 (8 days). Large mononuclear cells and polymorphonuclear leuko-
cytes in alveoli. $\times$ 300.

FIG. 4. Dog 17 (5 days). Plug of newly formed tissue in bronchus with metaplasia
of lining epithelium. $\times$ 105.

FIG. 5. Dog 25 (9 days). Plugs of organizing tissue in alveoli. $\times$ 300.
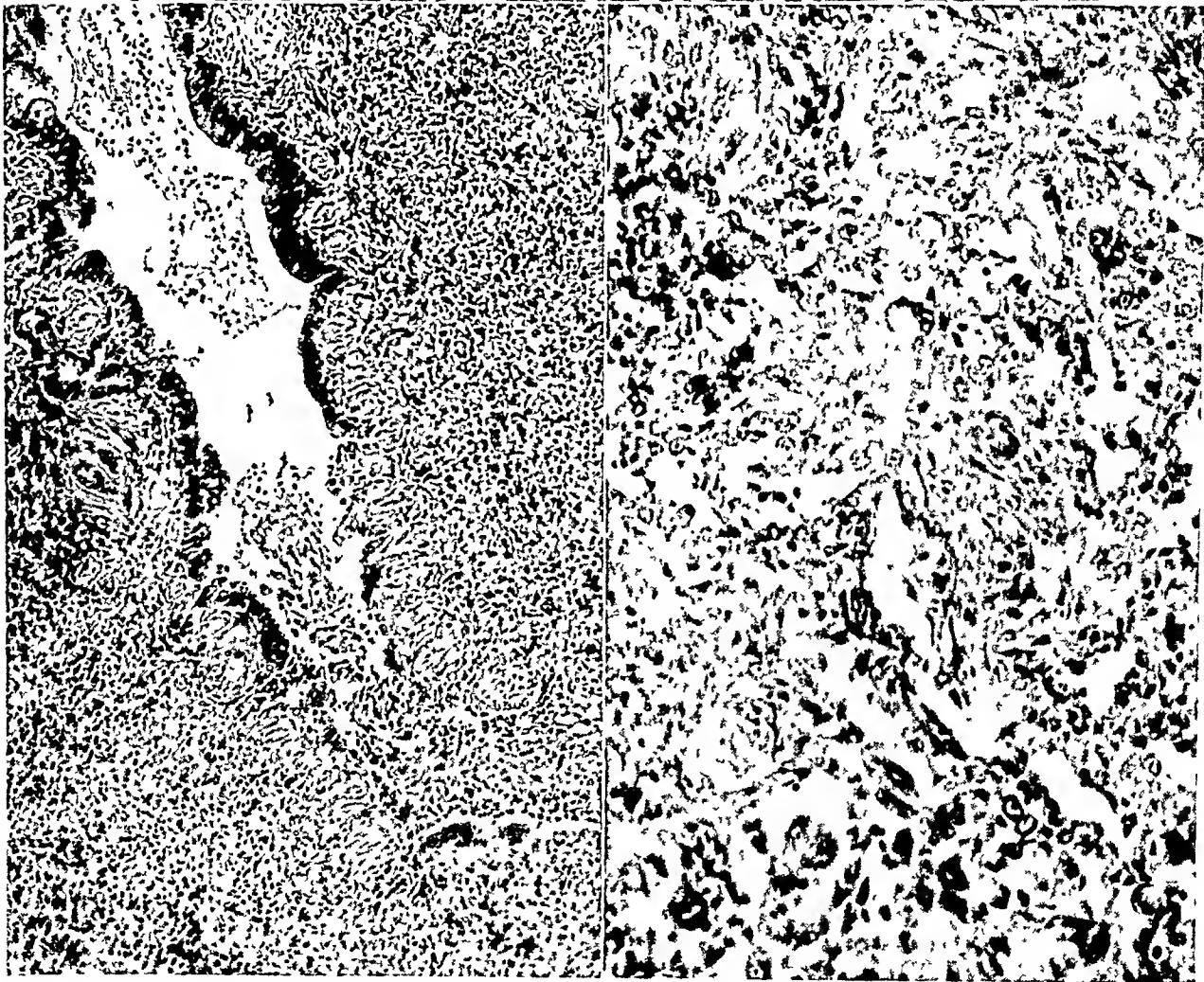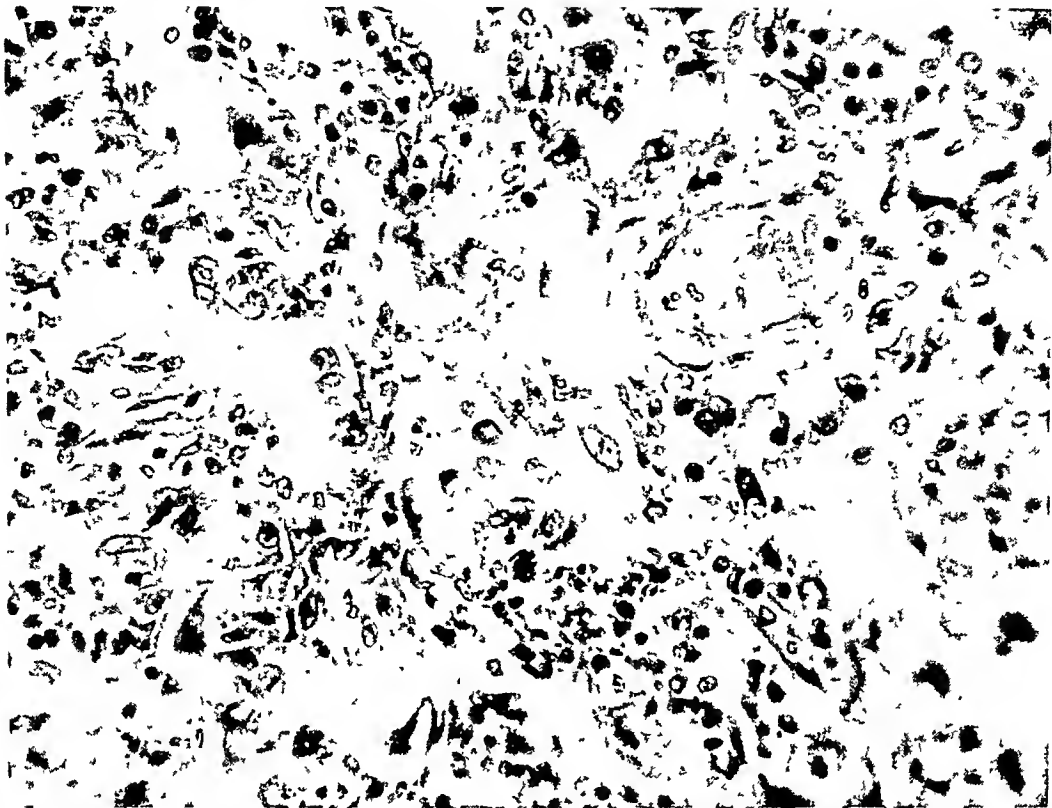
Durlacher and Bunting

Pulmonary Changes Following Phosgene

FIG. 6. Dog 21 (7 days). Bronchiole filled with fibroblastic tissue and proliferating epithelial cells. Thickening of walls of surrounding alveoli. $\times$ 220.

FIG. 7. Dog 27 (59 days). Re-epithelialized scar in bronchiole. $\times$ 100.

FIG. 8. Dog 27 (59 days). Subpleural atelectasis and scarring. Obliteration of bronchioles by scar tissue. $\times$ 45.
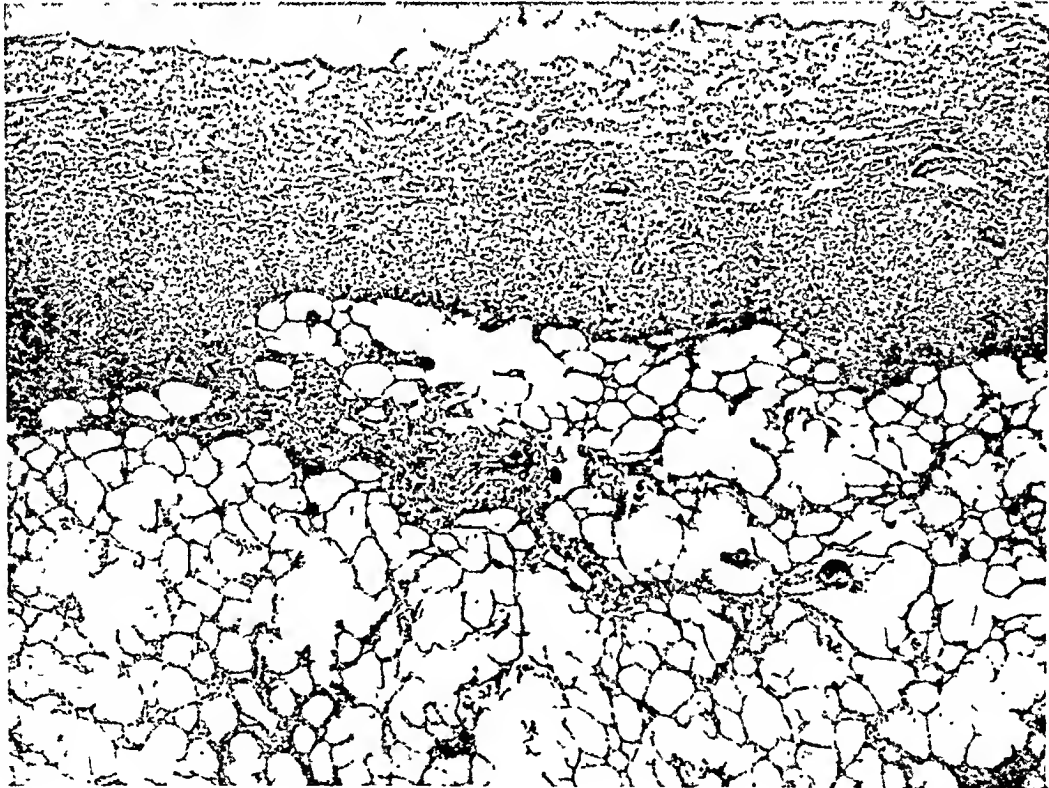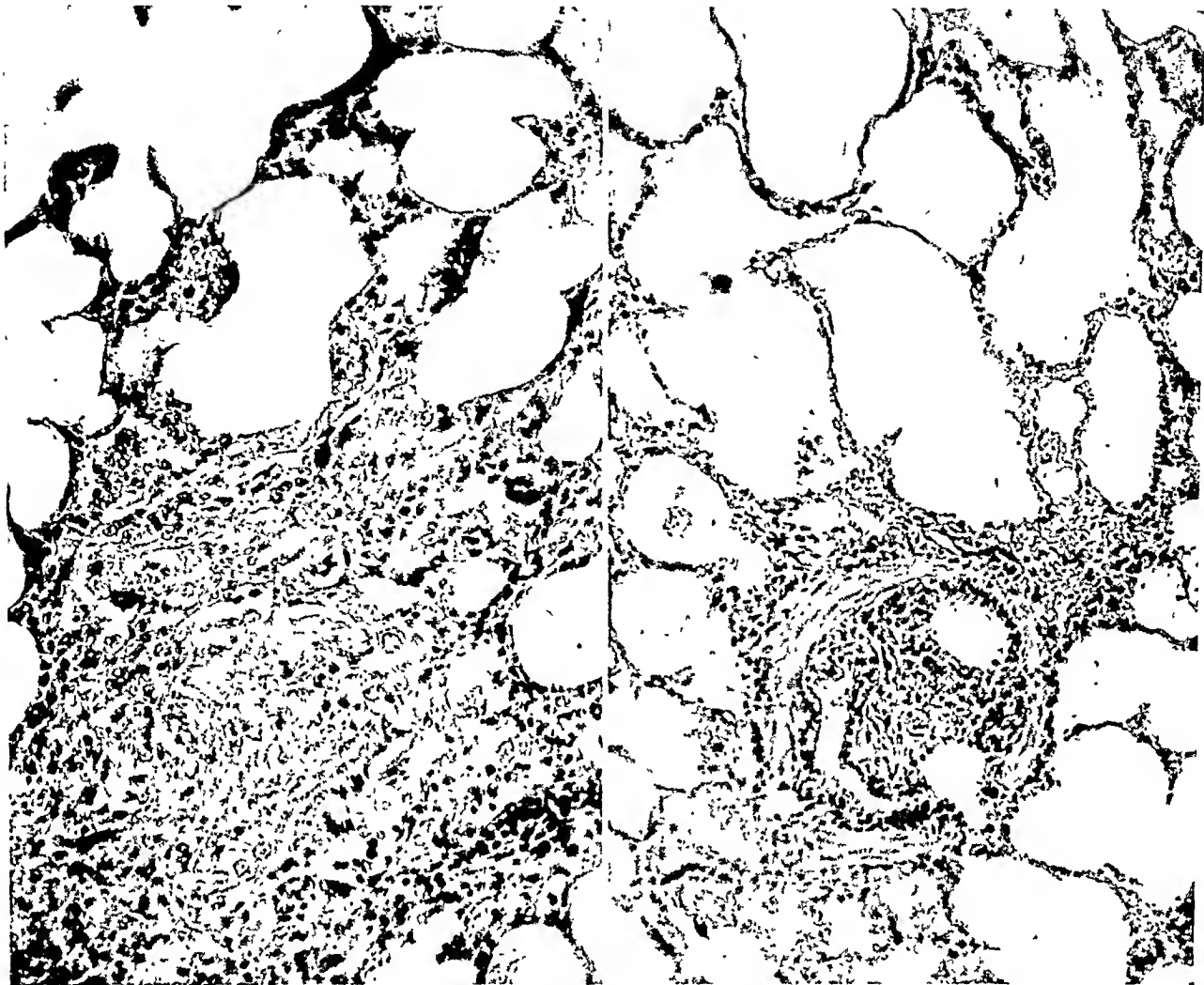
Durlacher and Bunting

Pulmonary Changes Following Phosgene

STUDIES OF THERMAL INJURY

II. The Relative Importance of Time and Surface Temperature in the Causation of Cutaneous Burns [*]

A. R. Moritz, M.D., and F. C. Henriques, Jr., Ph.D.

(From the Department of Legal Medicine, Harvard Medical School, Boston, Mass.)

Although it is common knowledge that there is an inverse relationship between the intensity of a thermal exposure and the amount of time required to produce a burn, there is remarkably little available information as to the rate at which burning of human skin occurs at any given surface temperature or as to the pathogenesis and pathological characteristics of burns in which the duration and degree of rise in intracutaneous temperature was known or could be calculated with any degree of accuracy.

Considerable information regarding the time-temperature thresholds at which cutaneous burning occurs in animals is provided by the experiments of Hudack and McMaster [1] and of Leach, Peters, and Rossiter.[2] In the former, water at temperatures ranging between 42° and 67°C. either was applied directly or was passed through a thin-walled glass chamber, the base of which was brought in contact with the skin of mice. In the experiments performed by Leach, Peters, and Rossiter water was pumped through a metal chamber at temperatures ranging between 45° and 80°C. and the base of the chamber was held in contact with the skin of guinea-pigs for varying periods of time. Both groups of investigators observed that the time required to produce injury diminished rapidly as the temperature of the water was raised. The former reported that a source temperature of 44°C. was critical for the causation of hyperthermic edema. The latter reported that the critical temperature for causing permanent and irreversible injury of guinea-pig skin lies between 50° and 55°C. Neither of the above-cited investigations provided data from which the time-temperature requirements for the production of burns of human skin could be estimated.

Although Leach, Peters, and Rossiter [2] made a careful study of the pathological characteristics of different kinds of burns of guinea-pig skin, the extent to which these changes are representative of those that occur in cutaneous burning in man was not disclosed.

The primary purpose of this investigation was to obtain information regarding the effects on human skin of episodes of hyperthermia of varying duration and of varying degrees of intensity. The direct approach would have been to make all experiments on human subjects. For various reasons this was not feasible. It was decided first to establish the time-temperature thresholds for varying degrees of cutaneous injury by experiments on an animal having a skin similar to that of man, and then by means of a relatively small number of critical exposures of human skin to establish the extent to which the more comprehensive animal data are applicable to man.

METHOD OF MAINTAINING SURFACE OF SKIN AT A KNOWN DEGREE OF HYPERTHERMIA

The method employed by Leach, Peters, and Rossiter [2] for the production of burns in guinea-pigs was investigated and found to be unsuited to the purposes of this study. It was discovered that the temperature of the stream of hot water flowing through the upper and midportions of the metal chamber was significantly and variably higher than that of the surface of the underlying skin. As the water flowed from inlet to outlet there remained a relatively static layer of fluid at the bottom of the chamber. Thus, there were interposed two hindrances to the conduction of heat between the site of measured temperature and the surface of the epidermis, one being the metallic base of the chamber and the other being the layer of quiet fluid above it. Thermocouple measurements of the temperature of the underlying skin disclosed it to be as much as 1° lower than that of the stream of water at the level of the thermometer. In consideration of the fact that the rate at which burning occurs is almost doubled for each degree rise in temperature between 44° and 51°C., the desirability of employing a more precise method of controlling the temperature of the skin is obvious. Another reason for rejecting the method used by Leach, Peters, and Rossiter for the production of burns was that the skin was compressed by the metallic base of the chamber during the period of heat transfer. It was our desire to investigate the effects of hyperthermia independently of any physiological artefact that might be introduced by compressive occlusion of dermal capillaries during the period of exposure.

Direct exposure of the surface of the skin to a rapidly flowing stream of hot liquid was chosen as the method best adapted for the acquisition of these data. With this type of exposure, the surface of the skin could be maintained at the temperature desired without the establishment of an appreciable gradient (<0.1°C.) between it and the source of heat. There was no insulation of the surface by a static

layer of gas, liquid or solid, no heat loss through vaporization of surface moisture, and no diminution of sub-surface heat conduction due to vascular occlusion by the application of pressure on the surface. The method was simple to operate and led to remarkably reproducible cutaneous effects.

The applicator by which a running stream of hot water was brought in direct contact with the skin consisted of a metal cup, the brim of which was covered with a pad of closed-cell sponge rubber to insure a watertight contact. By means of an electric pump, water was circulated from a large constant temperature reservoir through the cup, the open end of which was applied to the skin. The rate of flow was regulated by a screw clamp on the inlet tube and by the height of the outlet tube (Text-Fig. 1).
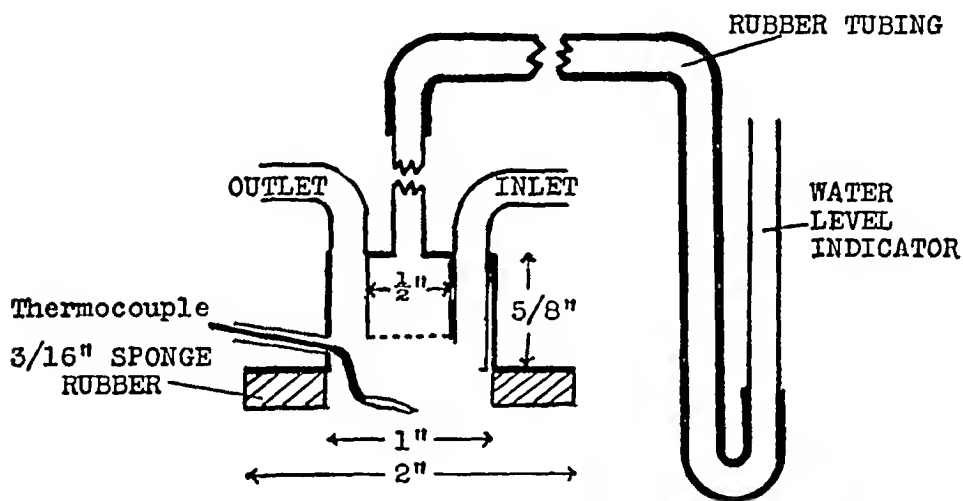
Text-Figure 1. An apparatus for exposing the skin to a flowing stream of liquid. The surface is brought immediately to, and maintained at, a predetermined and constant temperature without altering surface pressure. The apparatus consists of a brass cup, the base of which is open to permit direct contact between heat source and skin. Water (or oil) was heated by a manually operated steam coil in a large reservoir and pumped through the cup. The pressure within the cup was regulated by adjusting the rate of flow and the level of the outlet.

Tangential flow of a liquid produces no vertical component of force and thus no vertical pressure. Vertical water pressure within the cup could be varied between 70 and 86 cm. of mercury by suitable adjustments of the aperture of the inlet and the height of the outlet tubes. A copper-constantan thermocouple measured the temperature of the water flowing next to the skin. During any period of exposure the temperature of the water flowing over the skin could be controlled to within 0.1°C.

Two methods were used to equilibrate the apparatus before applying it to the skin. In one, the apparatus was applied to a block of linoleum, adjusted to the desired pressure, and transferred to the skin site to be exposed as soon as the temperature equilibrium was reached. In the

other, the applicator was allowed to remain immersed in the hot water reservoir with the pump turned on until thermal equilibrium was established. The cup was then transferred immediately to the skin and adjusted to the desired water pressure.

Provision was made in the construction of this apparatus for studying the relation of the size of the area of exposure to the intensity of the resultant injury. This was accomplished by making the brim of the cup removable so that the area of skin to be exposed could be varied according to the aperture selected for use. Thus, in the same region on the same animal and under identical conditions of time, temperature, and pressure, circular targets having a diameter of either 7 or 25 mm. could be exposed.

Individual burns in the animal experiments were 25 mm. in diameter. This was larger than was desirable for human subjects and the diameter of the aperture of the cup was accordingly reduced to 7 mm. for the human experiments. Before doing so, however, it was established by animal experimentation that the reduction in the size of the exposure area did not make an appreciable difference in the effect on the epidermis.

Water was employed as the source of heat in all of the experiments summarized in Table II. Because the question was raised whether a hypotonic fluid such as water might modify the effects of heat, a series of comparable exposures were made in which oil was substituted for water. There was no appreciable difference between the injury-producing potentiality of rapidly flowing streams of water and of oil on either animal or human skin so long as the temperature and duration of exposure were the same.

EXPERIMENTS ON PIGS

The pig was used in these studies because it was found that no other readily available animal has skin that bears so close an anatomical resemblance to that of man.

Porcine Epidermis

The epidermis over the lateral body area of the pig measures approximately 0.1 mm. in thickness. Like that of man there are irregularities in the contour of both the upper and lower surfaces of the epidermis, those on the upper being due to an intricate system of intercommunicating linear depressions and those on the lower corresponding to the dermal papillae over which the epidermis is moulded (Fig. 1 in Study III *).[3]

---

* Studies of Thermal Injury, III, will appear in the November issue of THE JOURNAL.

Like that of man, the outermost zone or stratum corneum of the pig's epidermis consists of several loosely connected layers of desiccated and intensely basophilic remains of keratinized epithelial cells.

The second or granular layer is thin and consists of several layers of dead or dying squamous cells, the acidophilic cytoplasm of which contains many fine, deeply basophilic keratohyaline granules. Many of these cells have lost their nuclei. Others contain shrunken hyperchromatic nuclei or Feulgen-negative nuclear ghosts.

The third zone is comprised of several layers of aging squamous cells which no longer have any direct cytoplasmic attachment to the dermis. The cytoplasm is dense, deeply acidophilic, and appears desiccated. The cells are so closely packed that neither intercellular bridges nor spaces can be recognized. Many of the nuclei are relatively small and more densely packed with chromatin granules than those of the deeper cells.

The fourth zone consists of cells in transition between the squamous and the basal cell layer. The transitional cells are large and polyhedral and many of them still have an attenuated foot-like cytoplasmic attachment to the dermis. It is in this zone that intercellular bridges of tonofibrils are most readily visualized. The cytoplasm is moderately basophilic. The cell outlines are distinct and the intercellular spaces are clearly defined. The nuclei are larger and rounder than those of the more superficial cells and contain several coarse and many fine granules of chromatin.

The fifth zone is comprised of the basal cells which, save for their cuboidal or columnar shape and their palisade-like arrangement on the dermis, are essentially similar to the overlying transitional cells. Projecting from the inferior surface of the basal epidermal cells of the pig are many robust tonofibrils which appear to be embedded in the dense feltwork of fine collagen fibrils that comprise the superficial zone of dermis. No such fibrillar anchorage of epidermis to dermis can be seen in human skin (Figs 1 to 6 in Study III).[3]

The microscopic appearance of the epidermis of both man and pig suggests that there is a progressive loss of intracellular water as the epithelial cells grow older and move away from the dermis. The nearer the surface the more desiccated the cells appear. The entire stratum corneum and most of the cells of the granular layer appear to be incapable of vital reaction.

Porcine Dermis

The dermis covering the lateral body surface of immature pigs measures between 1.0 and 2.0 mm. in thickness and is generally more compact than that of man. In both pig and man the superficial portion

of the dermis comprising the papillary layer or corium is characteristically a soft, thin, loosely arranged feltwork of delicate collagen fibrils in which there appears to be an abundant amount of interstitial fluid. In man it is readily distinguishable from the thick underlying reticular layer which is comprised of robust and closely interwoven bundles of collagen fibrils. Elastic fibrils are more numerous in human than in porcine skin. On the lateral body surface of the pig the corium tends to be thinner and less well defined than it is in man and in places is only slightly less compact than the reticular zone (Figs. 1 to 6 in Study III).[3] The deeper portion of the reticular connective tissue sends trabecular extensions into the underlying adipose hypodermis.

Blood Vessels of Porcine Skin

It was observed in ordinary histological preparations that the appearance of the capillaries in the dermal papillae of the body skin of the pig is similar to that in corresponding regions of man. In recognition of the fact that it is difficult or impossible to get an accurate impression of so complicated a structure as a capillary network by two-dimensional visualization, a modification of the Pickworth technic[4] was employed in order that the dermal blood vessels could be studied in three dimensions.

Maximum cutaneous hyperemia of an area of skin was induced by exposing it for 20 minutes to water at 50°C. After such an exposure the erythrocytes were so densely packed in the distended capillaries that there was practically no loss of blood when the skin was incised. Skin and subcutaneous tissue treated in this way was excised to a depth of 8 mm., fixed in 10 per cent formalin, cut in thick sections, and treated with benzidine.

The benzidine imparted a dark blue color to the contents of the engorged vessels. After skin treated in this manner was cleared, a three-dimensional study of its blood vessels could be made with a binocular microscope.

The appearance of the dermal vessels of porcine skin at various levels below the surface is shown in Text-Figure 2. To prepare this illustration a block of benzidine-treated skin was cut serially and parallel to the surface in sections measuring 50 μ in thickness. Another block of the same skin was cut serially and at right angles to the surface. Photographs were made of both series and the prints were mounted in such a manner as to orient the horizontal sections in relation to the depth below the surface that each represented.

In approaching the surface of the body, blood vessels follow an oblique course through the hypodermis and, after reaching the lower
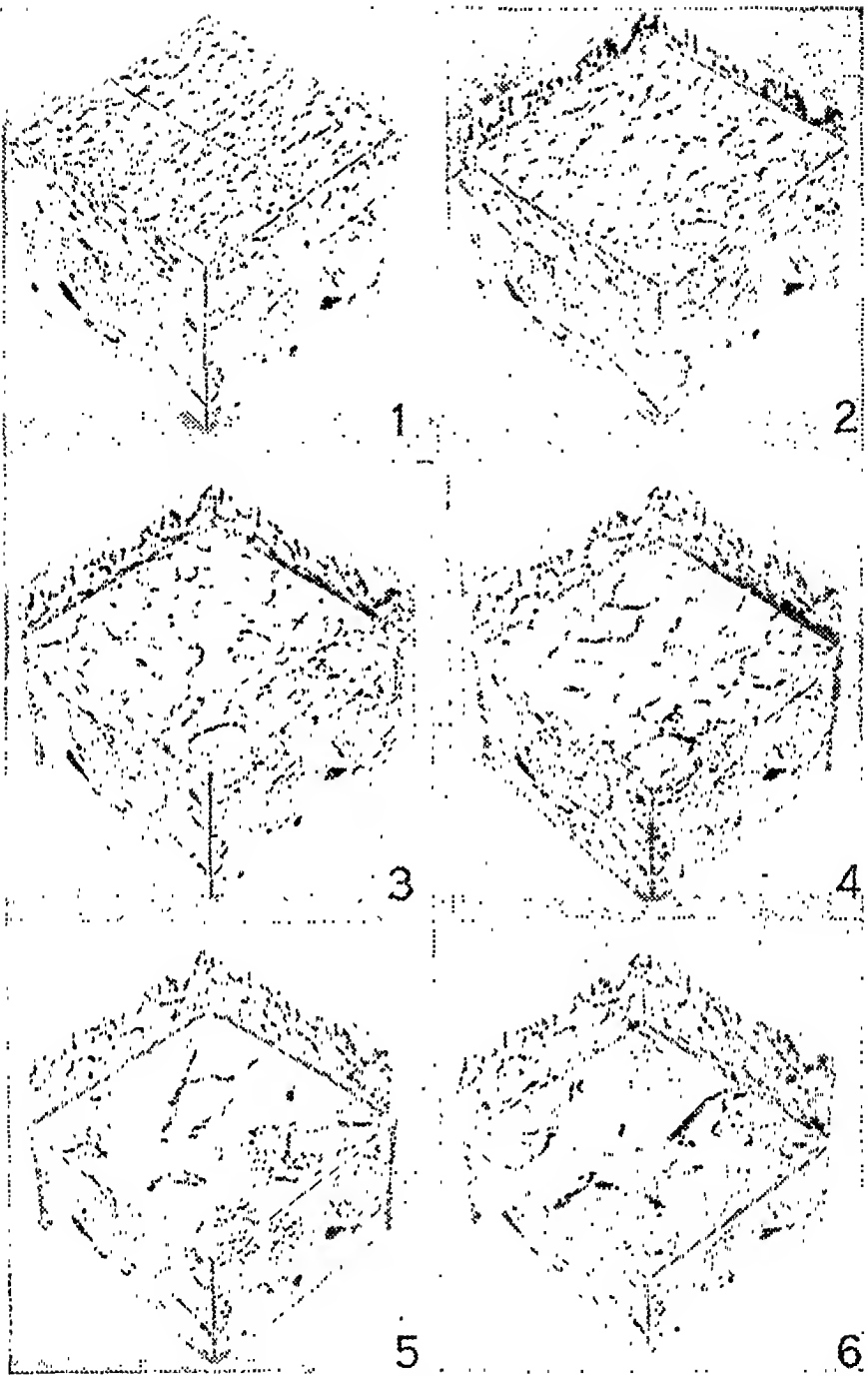
Text-Figure 2. Series of composite photomicrographs showing vascularization of a block of hyperemic porcine skin which measured 2 by 2 by 2 mm. A series of thick (50 μ) benzidine-treated horizontal and vertical sections were mounted in such a way as to show the distribution of veins, arteries, and capillaries at various levels beneath the surface. No. 1 shows the capillary plexus lying in the most superficial (50 μ) portion of the dermis. No 6 shows the vessels in the most superficial layer of the adipose tissue of the hypodermis.

layer of the dermis, branch horizontally to form multiple inter-venal and inter-arterial anastomoses. From these horizontal plexuses there originate a series of broad vascular loops that penetrate to the mid-

portion of the dermis. Inter-arterial and inter-venal anastomoses between these loops serve to establish a mid-dermal plexus. From this mid-dermal plexus originate numerous hairpin-shaped capillary loops which extend upward into the dermal papillae. These capillary loops anastomose freely with one another and constitute the most superficial or papillary plexus. The capillary communications between the superficial arterioles and venules occur at different levels. Some follow a course that brings them within a few micra of the basal epithelial cells over the tips of the papillae. Still others follow an almost horizontal course to establish communications between the arterioles and venules of the intermediate plexus. At all levels through the dermis there are numerous vascular communications with the mantle-like meshwork of capillaries that surrounds the hair follicles and dermal glands.

As may be seen in Text-Figure 2, the number, size, distribution, and communications of the dermal blood vessels of the pig are remarkably similar to those described by Spalteholz[5] in human skin. The similarity of blood vessels in human and porcine skin was found to be so great that it was with difficulty that one could be distinguished from the other in Pickworth preparations.

It is not intended to imply that the anatomical resemblance between the vessels of human and porcine skin denotes an equal degree of functional similarity. Certainly, the vascularization of both indicates that ample and similar mechanical facilities exist either for the transfer of body heat to the surface to facilitate its dissipation, or for the conduct of surface heat to the interior to raise the internal temperature of the body.

Sweat Glands and Sweating

Several types of glands are encountered in the dermis of the pig and although one of them bears some resemblance to the sudoriferous glands of human skin, it does not secrete a significant amount of sweat.

The fact that the pig does not sweat was verified by a series of experiments in which the water loss from the skin of living pigs was measured at various environmental temperatures, with and without the administration of pilocarpine (Table I).

It was found that the water loss from the skin of a live pig does not differ significantly from that of one that is dead. In a cool environment the water loss per square cm. per minute is approximately the same in man and pig. At higher environmental temperatures the rate of water loss from human skin is tremendously augmented, whereas the corresponding increase in water loss from the skin of a pig is relatively small and is due to more rapid evaporation of tissue water rather than to sweating.

So far as can be judged by anatomical criteria, the pig should be a suitable experimental subject from which to derive certain types of information regarding the effects of heat on human skin. Its various layers are of comparable thickness and structure. Its blood vessels are similar in size, number, and distribution. As will be shown later, its susceptibility and reactions to control episodes of hyperthermia are remarkably similar to those of man.

TABLE I

Rate of Water Loss from Surface of Human and Porcine Skin *

| | Water loss (mg. per sq. cm. per minute) during a period of 10 minutes | | | | | | | |
| | Temp., 21°C.; humidity, 30 to 40% | | | | Temp., 36°C.; humidity, 30 to 40% | | | |
	No. of tests	Mini-mum	Maxi-mum	Mean	No. of tests	Mini-mum	Maxi-mum	Mean
Dead pig (lateral thoracic region)	4	0.016	0.026	0.019	4	0.023	0.031	0.027
Live pig (lateral thoracic region) without pilocarpine	5	0.016	0.028	0.021	6	0.020	0.032	0.028
Live pig (lateral thigh):								
Without pilocarpine					4	0.018	0.026	0.024
†With pilocarpine (1 mg. per kg. of body weight)					4	0.021	0.030	0.027
Live man (forearm):								
Subject #1 (A.R.) without pilocarpine	1			0.027	1			0.180
Subject #2 (A.M.) without pilocarpine	2	0.028	0.038	0.033	2	0.280	0.360	0.320

* Amount of water loss was determined by accretion in weight of $Mg(ClO_4)_2$ contained in base of weighing bottle during the time that the neck of the bottle was held in contact with the skin.
† Iodine color test negative.

Since a pig does not sweat, allowance should be made for the inability of porcine skin to lose heat through the vaporization of moisture derived from sweating. The significance of heat loss through vaporization of moisture in respect to cutaneous burning will be discussed in greater detail in study IV of this series.[6]

Thermal Exposures of Porcine Skin

Closely clipped young (8 to 10 kg.) white pigs were used. It was found that the skin of the pig was not uniformly susceptible to thermal injury. That covering the ears, thighs, buttocks, and ventral surface was more, and that of the neck and midportion of the back less vulnerable, than was the skin of the lateral portion of the shoulders, thorax, and abdomen. The largest uniformly reacting area was the lateral body surface beginning anterior to the thighs and extending forward over the shoulders.

TABLE II

Time-Surface Temperature Thresholds for Thermal Injury of Porcine Skin

Column groupings: columns "Mild", "Severe", "Scaling", "Small ulcers" fall under **Sub-threshold exposures (1° reactions)** — "Mild" and "Severe" under *Hyperemia only*; "Scaling" and "Small ulcers" under *Focal epidermal necrosis*. Columns "Red burn" and "Pale burn" fall under **Threshold and supra-threshold exposures (2° and 3° reactions)** — *Complete epidermal necrosis*.

Temperature in °C.	Time: Minutes	Time: Seconds	Number of experiments	Mild	Severe	Scaling	Small ulcers	Red burn	Pale burn
44	420		1	X				X	
45	150		1	X					
45	180		1					X	
46	45		1	X					
46	60		1		X				
46	90		1					X	
46.5	45		1	X					
46.5	60		1					X	
47	35		1	X					
47	45		1		X				
47	50		1					X	
47	60		1					X	
48	10		3	X					
48	12		1		X				
48	14		2		X				
48	14		1			X			
48	15		2					X	
48	16		1					X	
48	18		1					X	
48	20		1					X	
52 Cont.	2		4					X	
52 Cont.	3		1					X	
53		20	1	X					
53		30	1		X				
53		45	2			X			
53	1		2					X	
53	1	30	3					X	
53	2		1					X	
54		15	1	X					
54		25	1		X				
54		35	1					X	
55		5	1	X					
55		10	1	X					
55		15	1	X					
55		20	1			X			
55		25	1					X	
55		30	3					X	
56		10	1	X					
56		15	1			X		X	
56		20	1	X				X	

58	60		65	70	75	80	85	90	95	100
				· X	X	X	X	X	X	X
X		X	X X X	X X X	X X	X	X	X	X	X
		X								
		X X								
X	X		X							
1 1	1 1 1 1 1 1 2 1		1 1 1 1	2 1 2	1 1	1 1	1 1	1 1	1 1	1 1
5 10	2 2 3 5 7 7 10		1 2 3	1 2	1 5	1 5	1 5	1 5	1 3	1 3
		10	10	3						

49		50		51		52
	X X X		X X X		X X X X	X
	X X X		X		X	
	X X X		X X		X X	
	X X		X		X	X
X X X		X X		X X X		X
4 5 2 5 2 2 2 1 1 4 1 2 1 1 5		1 1 1 1 3 2 2 2 2		2 2 2 1 2 2 2 2 1 1 2		1 1 1
			30 45 30			30 45 30
3 4 5 6 6 6 7 7 7 8 8 8 9 10		1 2 4 5 5 5 5 6 6		1 1 2 3 3 3 4 5 5 10		1

The results of 179 exposures of pigs' skin with temperature and duration of each are shown in Table II. All animals were first anesthetized by intraperitoneal injection of pentobarbital sodium.

The surface temperatures at which these exposures were made ranged between 44° and 100°C. The duration of exposures varied between 1 second and 7 hours. The majority of the exposed sites were kept under observation until the reaction had subsided or the lesion had healed. In the case of borderline reactions, duplicate exposures were made and the areas excised at the end of 24 or 48 hours for microscopical study. As indicated in Table II, a wide variety of reactions was observed. These ranged in severity from evanescent erythema to deep necrosis.

It was found that all exposures fell into one of two groups according to whether they had caused full-thickness destruction of the epidermis over the entire target area. Those that failed to cause complete transepidermal necrosis were designated as sub-threshold. Those that resulted in complete trans-epidermal necrosis were designated as threshold or supra-threshold depending on whether they were just sufficient or more than sufficient to destroy the epidermis.

Reactions to exposures that were of insufficient intensity or duration to cause complete destruction of the epidermis were designated as first degree. In the mildest of these, the total response to the episode of hyperthermia was evanescent dilatation of superficial cutaneous blood vessels. In others, the hyperemia was more intense and prolonged. In still others, the occurrence after a few days of excessive exfoliation or focal ulceration indicated that some of the exposed epidermis had sustained irreversible injury.

Cutaneous reactions indicative of full-thickness destruction of epidermis over the entire target area were designated as second or third degree according to the depth to which irreversible injury was estimated to have occurred. If the clinical course or microscopic appearance of a lesion indicated that trans-epidermal necrosis had occurred without a significant amount of irreversible damage to the dermis, the reaction was designated as second degree. The more any given exposure exceeded in either duration or intensity the threshold at which the epidermis was destroyed, the greater the depth to which the dermis was affected. Reactions indicating that a significant degree of irreversible injury to the dermis had occurred were designated as third degree. In all second and in many third degree reactions the burned skin was visibly hyperemic for many days. In some third degree reactions the surface of the burn became immediately ischemic and re-

mained so until the pale and necrotic layer of superficial tissue was detached.

In the beginning there was some difficulty in the establishment of clinical criteria by which to predict the ultimate severity of certain injuries. Although there was no difficulty in recognizing almost immediately the difference between a reaction of which the total effect was a mild and transient erythema and one that consisted of deep coagulation necrosis, it was not always possible during the first few days to recognize by clinical observations whether a given lesion represented a severe first degree reaction with incomplete or focal epidermal destruction or a relatively mild second degree reaction.

Apart from the microscopic appearance, the most reliable criteria by which to recognize trans-epidermal necrosis were (a) the ease with which dead but still intact epidermis could be displaced by friction on the second and third days after exposure, and (b) the development of complete encrustation of such a lesion within a week.

The macroscopic appearance of different degrees of cutaneous reaction to hyperthermia may be seen in the photographs of the right and left sides of pig 924 in Text-Figure 3, made when the lesions on the right side were 24 hours old and those on the left were 7 days old. It is apparent from these photographs that the duration of exposure at any given temperature was remarkably critical in relation to the kind of reaction evoked. It is equally apparent that the time required to produce a given degree of reaction varied inversely with the temperature.

EXPERIMENTS ON HUMAN SUBJECTS

In order to determine the extent to which the results of experiments on pigs were applicable to man, a series of 33 exposures were made on human volunteers (Table III). In some the heat was applied to the skin of the anterior thoracic region and in others to the ventral aspect of the forearm. The exposures were made with the apparatus shown in Text-Figure 1.

As in the pig, the reactions of human skin to hyperthermia were designated as first, second, or third degree. Reactions characterized as first degree were those in which part or all of the epidermis escaped irreversible damage. At one extreme a first degree reaction consisted of nothing more than transient hyperemia. At the other, the erythema was more severe and prolonged and was followed by the formation of miliary vesicles which did not coalesce. Lesions in which there was complete necrosis of the epidermis over the entire target area were designated as second or third degree reactions, depending on the depth
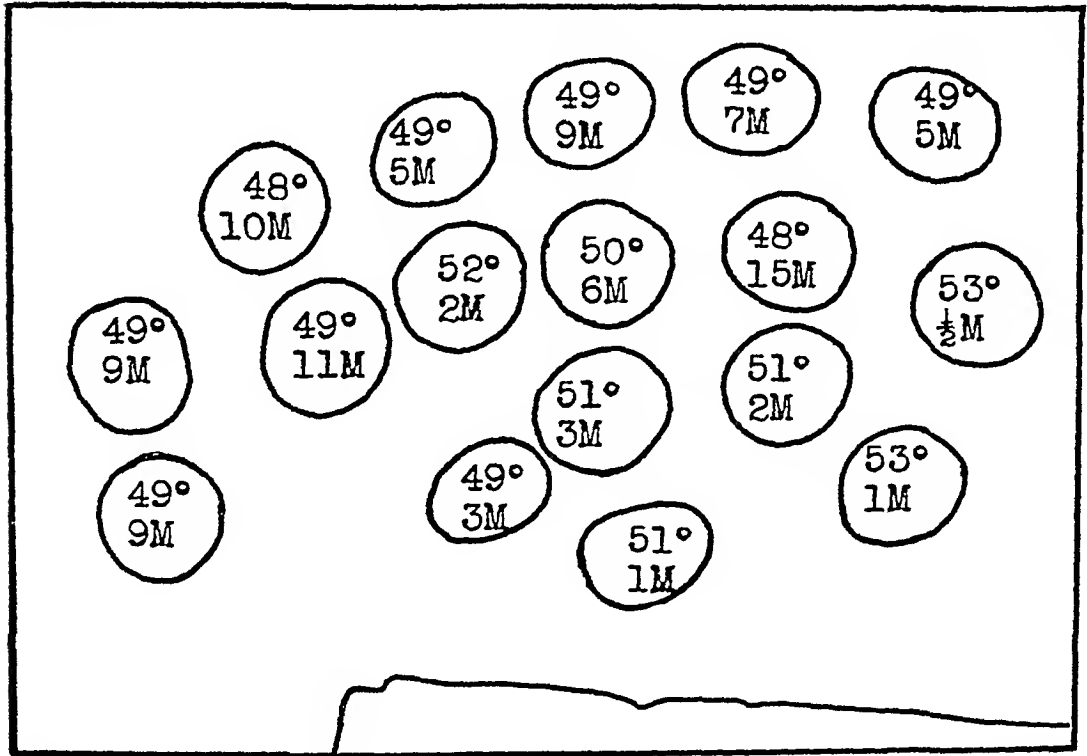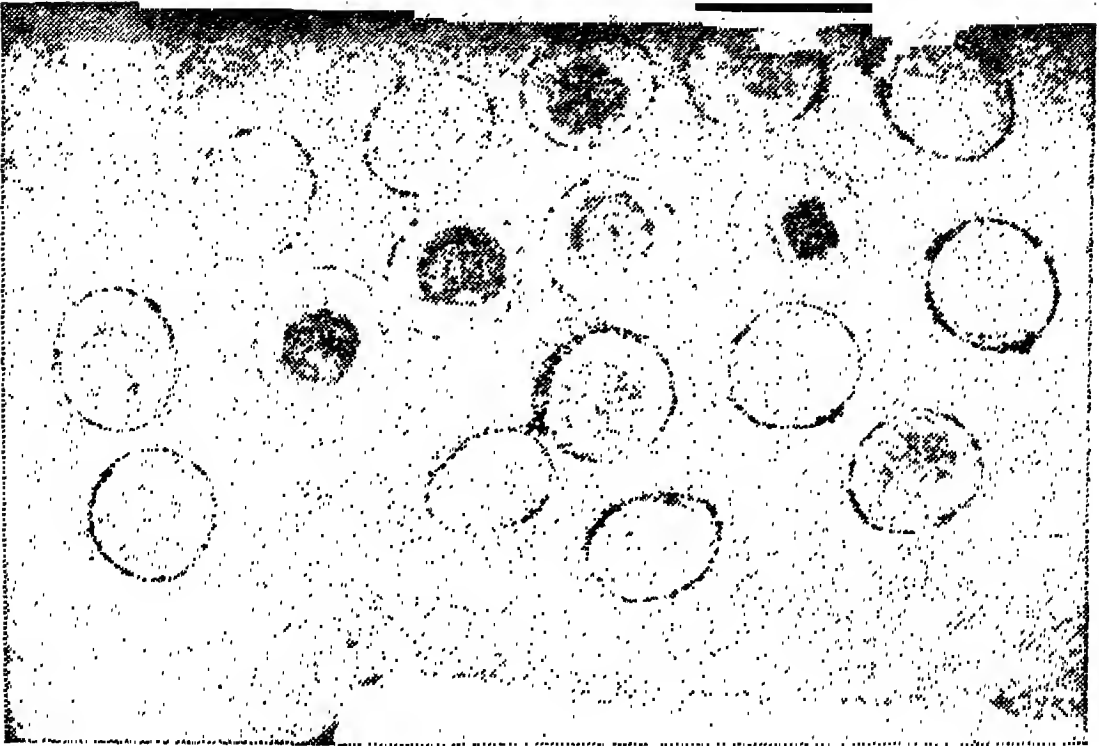
Text-Figure 3-a. Photograph and diagram of burns on the left side of a pig, with the temperature and duration of exposure indicated. Lesions are 7 days old.

to which the dermis appeared to have been destroyed. As in the experiments on pigs, a threshold exposure represented the shortest time at any given temperature that caused complete destruction of the epidermis.
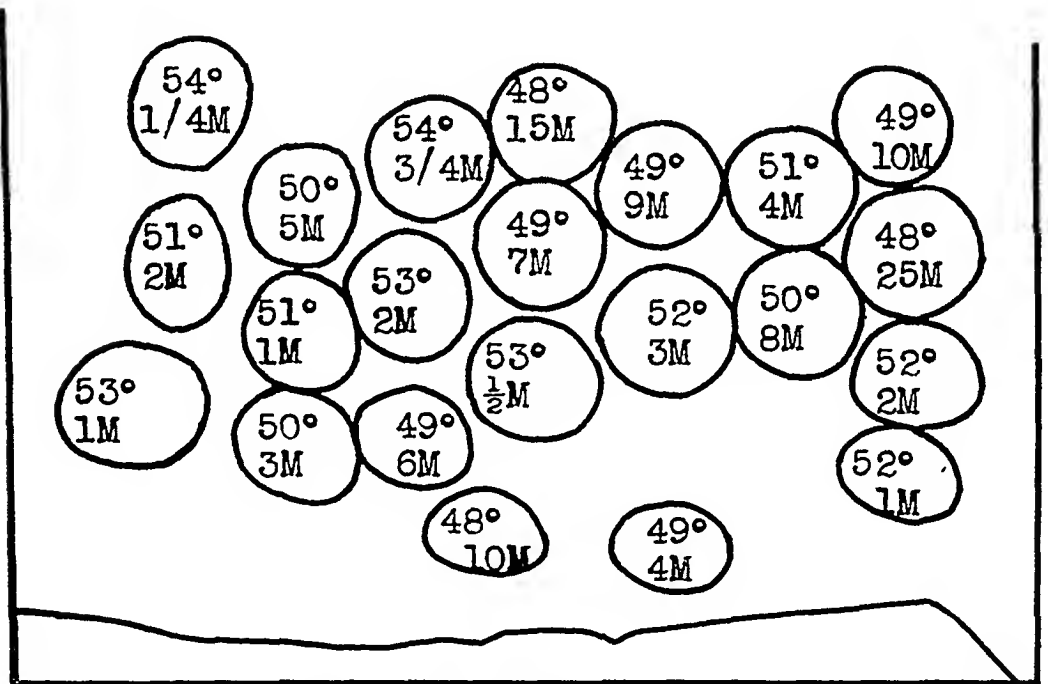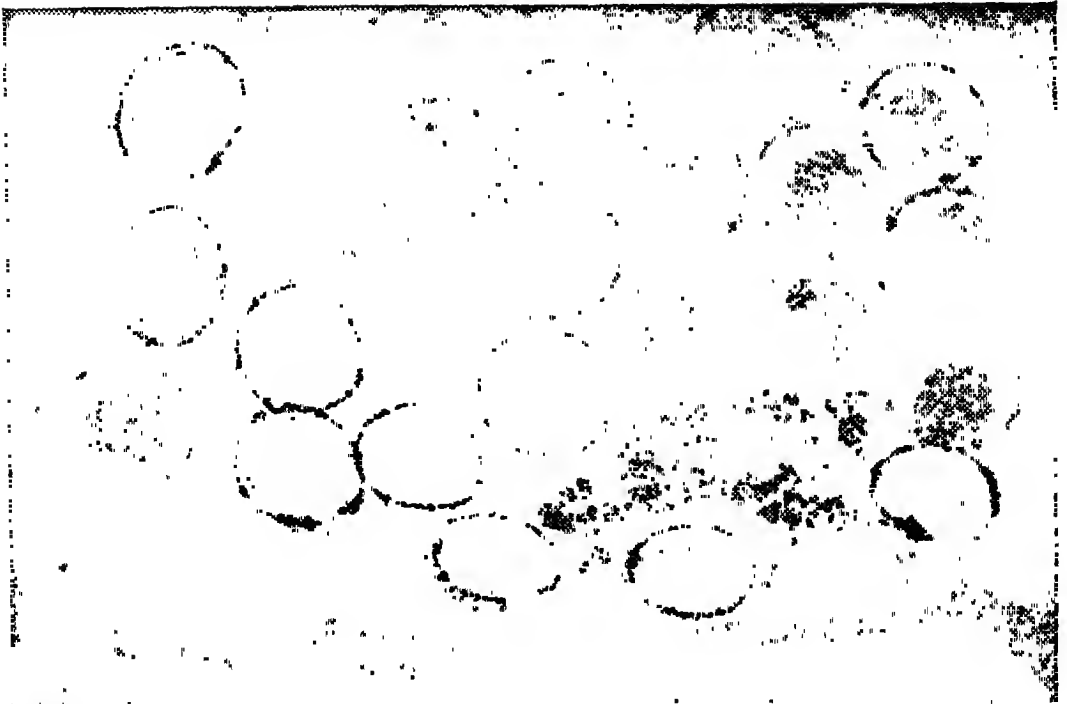
Text-Figure 3-b. Photograph and diagram of burns on the right side of a pig, with the temperature and duration of exposure indicated. Lesions are 24 hours old.

That a given exposure of human skin had resulted in trans-epidermal necrosis was usually, but not always, recognized by early and complete vesication of the target area. Although vesication indicated that the epidermis had been destroyed, absence of vesication did not always indicate epidermal survival. In several instances trans-epidermal necrosis occurred without vesication after supra-threshold exposures.

The explanation of this phenomenon will be discussed subsequently in relation to the pathogenesis of burns.

Discomfort in the form of a stinging sensation occurred between 47.5° and 48.5°C. and was felt more intensely by some subjects than

TABLE III

Time-Surface Temperature Thresholds for Thermal Injury of Human Skin

No.	Temp. at surface in °C.	Duration of exposure			Sub-threshold exposures 1° reactions Hyperemia without loss of epidermis	Threshold and supra-threshold exposures 2° and 3° reactions Complete epidermal necrosis	Subject	Date
		Hours	Minutes	Seconds				
1	44	5			x		BF	2/6
2*		5			x		BF	2/23
3		6				x	BF	2/6
4*		6				x	BF	2/23
5*	45	2			x		KL	2/16
6*		3				x	KL	2/3
7		3				x	HA	2/4
8*	47		18			x	RK†	2/13
9*			20		x		KL	2/25
10*			20		x		AM	2/26
11*			20		x		PG	2/26
12			25			x	RK†	1/8
13*			40			x	AM	2/26
14			40			x	PG	2/26
15			45			x	RK†	1/8
16	48		15		x		PG	7/19
17			15			x	AR	7/19
18			18			x	AM	6/26
19*	49		8		x		AM	2/16
20			8		x		AM	6/26
21			9	30		x	AM	6/26
22*			10			x	AM	6/26
23			11			x	AM	6/26
24			15			x	AM	6/26
25	51		2		x		AM	6/26
26			4			x	AM	6/26
27			6			x	AM	6/26
28	53			30	x		AM	6/26
29			1	30		x	AM	6/26
30	55			20	x		PG	7/19
31				30		x	AR	7/19
32*	60			3	x		FH	2/1
33*				5		x	FH	2/1

* Oil used instead of water as source of heat.
† Subject RK was atypical in that his threshold for thermal injury was significantly lower than that of other experimental subjects.

by others. Severe burns were sustained without discomfort at 47°C. and intense discomfort was sometimes complained of before any irreversible injury had been sustained at temperatures in excess of 48°C.

The results of the human experiments have been summarized in Table III.

Relative Vulnerability of Porcine and Human Skin to Thermal Injury

To facilitate comparison of the data included in Tables II and III, certain of the more critical observations in both have been combined graphically in Text-Figure 4. The solid line was established by points representing the time and temperature of exposures that caused minimal, second degree reactions of porcine skin. The points by which this
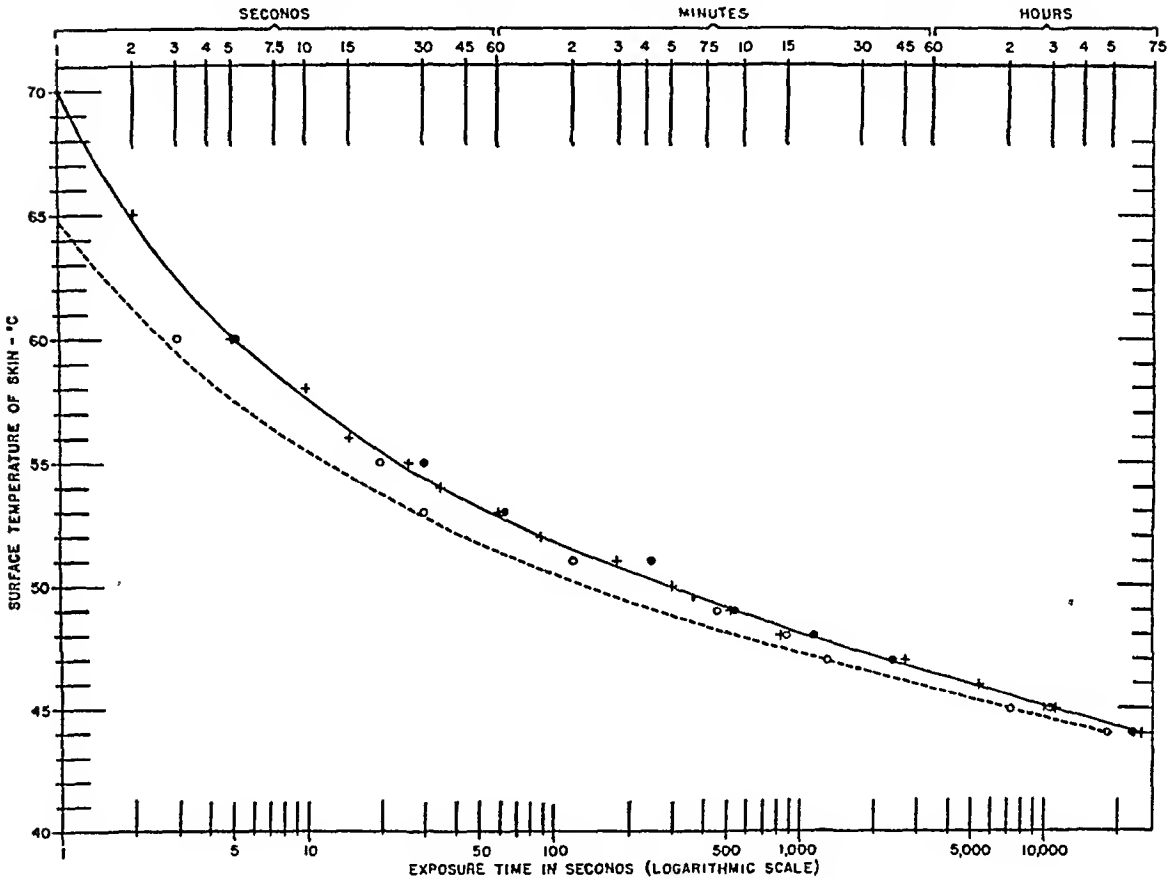
Text-Figure 4. Time-surface temperature thresholds at which cutaneous burning occurs. The broken line indicates the threshold at which irreversible epidermal injury of porcine skin is first sustained. The solid line indicates the threshold at which epidermal necrosis of porcine skin occurs. Critical exposures of porcine skin are represented by crosses. Each cross denotes the shortest exposure time at the temperature indicated which resulted in trans-epidermal necrosis. The results of critical experimental exposures of human skin are indicated by circles. The open circles represent the longest exposure at the temperature indicated that failed to destroy the epidermis, and the solid circles represent the shortest exposure at the temperature indicated that resulted in trans-epidermal necrosis.

line was established are represented by crosses. Each cross represents the shortest time at the temperature indicated that resulted in transepidermal necrosis of the entire target area afer exposure of pig's skin. The more that the time of any given exposure placed it to the right, or that the temperature placed it above the solid line, the greater the

depth to which the skin was destroyed. All exposures that were situated a significant distance above and to the right of the solid line were supra-threshold and all those situated a significant distance below and to the left of the solid line were sub-threshold.

The extent to which the reactions of human exposure corresponded to those observed in the more comprehensive animal experiments is indicated by the open and solid circles in Text-Figure 4. The open circles represent the maximum exposures that failed to destroy human epidermis and the closed circles represent the minimum time at the temperature indicated that resulted in complete destruction of human epidermis.

The broken line in Text-Figure 4 represents the approximate threshold at which the first morphological evidence of thermal damage to porcine epidermis was recognized. Exposures situated below the broken line caused no appreciable injury. Exposures lying between the broken and solid lines resulted in varying degrees of epidermal damage short of trans-epidermal necrosis. Since the reaction of human skin to controlled episodes of hyperthermia was not examined microscopically, no inferences can be drawn as to the precise time at any given temperature at which microscopic evidence of injury to human epidermis was first recognizable.

The results of the two sets of experiments (Tables II and III) indicate that at similar surface temperatures there is little or no quantitative difference in the susceptibility of human and porcine epidermis to thermal injury. The time-surface temperature threshold for the occurrence of trans-epidermal necrosis in man appears to be similar to that for the pig. It may be inferred that the optimal thermal milieu of the epidermal cells of both man and pig lies within a few degrees of the temperature that is normal for their internal tissues and that any rise in epidermal temperature above that level may be injurious if sufficiently prolonged.

The lowest surface temperature that was responsible for cutaneous burning in these experiments was 44°C. and the time required to cause irreversible damage to epidermal cells at this temperature was approximately 6 hours. It could be inferred from the contour of the curve (Text-Fig. 4) which represents the injury-producing threshold that burning would probably have occurred at even lower temperatures if the experiments had been sufficiently prolonged. The rate at which irreversible cellular injury was sustained increased rapidly as the surface temperature was raised, and for each degree rise in surface temperature, between 44° and 51°C., the time required to produce such injury was reduced by approximately one-half.

Above 51°C. the rate of injury began to fall off and the time-temperature curve depicting the threshold at which trans-epidermal necrosis occurred tends to become asymptotic in relation to the temperature axis. Below 44°C. there was a rapid decrease in the rate at which burning occurred and the time-temperature curve depicting the threshold for burning becomes asymptotic in relation to the time axis.

Through reference to Text-Figures 1 and 2 in Study III,[3] it will be apparent that the change in trans-epidermal temperature caused by exposing the surface of the skin to excessive heat is comprised of two phases. The first represents the time required to satisfy the thermal capacity of the epidermis or the transfer of a sufficient amount of heat energy to establish a stabilized trans-epidermal gradient. Thirty seconds was ordinarily sufficient for the attainment of a steady state of heat transfer in which the temperature at the basal cell level was only slightly lower than that at the surface. The second phase represents that part of the hyperthermic episode in which the trans-epidermal temperature gradient was stabilized.

Thus, in the case of surface temperatures under 51°C. the time required to cause irreversible injury of the epidermis was so long in relation to the amount of time required to bring the temperature of the basal cell level to a steady state that the latter was negligible. The total exposure time required to destroy the epidermis at such surface temperatures was essentially identical to the total duration of the steady thermal state within the epidermis, and under these circumstances there was a linear relationship between time and surface temperature in the production of burns between 44° and 51°C.

The reason that this linear relationship did not prevail below 44°C. probably was due to the increased effectiveness of the cellular reparative processes as the hyperthermic level approached the temperature range that was normal for the tissue.

As surface temperature rose above 51°C. and the total periods of exposure were shortened, the 30 seconds required to stabilize the epidermal temperature came to represent a progressively larger proportion of the entire hyperthermic episode. There was no longer the same kind of relationship between the surface temperature and that at the basal cell level as existed with the longer exposures and there was a progressive deviation from the linear relationship of surface temperature and time that characterized the injury curve between 44° and 51°C.

It should be borne in mind that these data refer to surface rather than to environmental temperature and it is not intended to imply that identical circumambient temperatures necessarily result in identical

surface temperatures of human and porcine skin. The only inference that is justified is that at any given surface temperature the time required to destroy porcine epidermis is approximately the same as that required to destroy human epidermis.

A mathematical analysis of these and other data and a consideration of their significance in relation to the rate processes of other physico-chemical phenomena are included in Study V of this series.[7]

VULNERABILITY OF ISCHEMIC SKIN TO THERMAL INJURY

One of the reasons that exposure of the skin to a running stream of hot water was the method of choice in these experiments was the belief that by this technic there would be no mechanical interference with the circulation of blood through the dermal capillaries. All of the foregoing exposures were made at atmospheric pressure. It was believed that circulation of relatively cool blood through the dermal capillaries probably would tend to protect the skin against burning and that to be applicable to field conditions data on the tolerance of skin to hyperthermia should be derived from the reactions of physiologically normal tissue.

In order to determine the extent to which local impairment in blood flow may increase the vulnerability of the epidermis to thermal injury, the following experiments were undertaken.

A control series of burns was made on each of 3 pigs by exposing various skin sites to running water at atmospheric pressure. The predetermined time and temperature of each exposure was such that severe first degree or mild second degree reactions could be anticipated (Table IV).

It was found that all 7 minute exposures at 49°C. and all 2 minute exposures at 51°C. made at atmospheric pressure were sub-threshold in the sense that they failed to cause complete trans-epidermal necrosis. That they were close to threshold was indicated by the fact that all 9 minute exposures at 49°C. and all 4 minute exposures at 51°C. did cause trans-epidermal necrosis.

Having established the position of the threshold for trans-epidermal necrosis in these animals to be between 7 and 9 minutes at 49°C. and between 2 and 4 minutes at 51°C. for exposures made at atmospheric pressure, a second series of exposures was now made in which the water pressure was increased by an amount corresponding to 80 mm. of mercury. With this pressure on the surface of the skin during the time that it was exposed to heat, there was no instance in which the reaction to a 7 minute exposure at 49°C. or to a 2 minute exposure at 51° C. was increased in severity.

It is apparent from the data summarized in Table IV that the application of pressure sufficient to collapse superficial dermal capillaries during a period of exposure does not cause appreciable augmentation in the vulnerability of epidermis to thermal injury.

In view of the extreme thinness of the epidermis, these results were to be expected. For reasons discussed in Study I of this series,[8] the temperature of the basal cell layer of the epidermis is determined primarily by the temperature of the surface. Thus, the dermal tem-

TABLE IV

Effect of Thermal Exposures with and without Pressure Ischemia

Animal no.	Tempera-ture	Duration of exposure	Excess pressure on skin	No. of exposures made	Number of lesions	
					Without trans-epidermal necrosis	With trans-epidermal necrosis
	(°C.)	(minutes)	(mm. Hg)			
887	49	7	0	5	5	0
	49	9	0	5	0	5
	49	7	86	5	5	0
899	49	7	0	4	4	0
	49	8	0	4	2	2
	49	9	0	4	0	4
	49	7	80	4	4	0
	49	8	80	4	3	1
901	51	2	0	3	3	0
	51	3	0	3	2	1
	51	4	0	3	0	3
	51	2	80	3	3	0
	51	3	80	3	1	2

perature gradients, which may be appreciably altered in ischemic as compared to normal skin during thermal exposure, would have little effect on the time-temperature relationship that exists at the epidermal-dermal interface.

LATENT THERMAL INJURY AND THE CUMULATIVE EFFECTS OF REPEATED SUB-THRESHOLD EXPOSURES

When the data summarized graphically in Text-Figure 4 are recalled, it is apparent that morphologic cellular alterations occurred only during the terminal phase of sub-threshold exposures. Not until the duration of any given episode of hyperthermia was such as to bring it to the level indicated by the interrupted line in Text-Figure 4 was there recognizable evidence of epidermal injury. This phenomenon is even more readily apparent in the photographs shown in Text-Figure 3. In these it may be seen that the 7 minute exposure at 49°C. on the left side of the animal shows only a trace of residual erythema whereas

TABLE V

The Cumulative Effects of Repeated Sub-Threshold Thermal Exposures * *on the Skin of the Pig*

Duration of each exposure	No. of exposures at same site	Interval between exposures	Effect of exposure on skin				Reference no.
			No evidence of epidermal injury		Epidermal necrosis		
			Mild vascular reaction	Severe vascular reaction	Focal	Complete and irreversible	
(minutes)							
3	1		x				1
3	1		x				2
3	1		x				3
4	1		x				4
5	1		x				5
6	1			x			6
6	1			x			7
6	1			x			8
7	1			x			9
7	1				x		10
8	1				x		11
8	1				x		12
8	1					x	13
9	1					x	14
9	1					x	15
9	1					x	16
9	1					x	17
9	1					x	18
3	3	3 min.				x	19
3	3	3 min.				x	20
3	3	3 min.				x	21
3	3	6 min.				x	22
3	3	12 min.				x	23
3	3	24 min.				x	24
3	3	48 min.				x	25
3	3	48 min.			x		26
3	3	72 min.			x		27
3	3	72 min.			x		28
3	3	96 min.			x		29
3	3	120 min.		x			30
3	3	240 min.	x				31
3	3	24 hrs.	x				32
3	3	48 hrs.	x				33
2	5	2 min.				x	34
2	5	30 min.	x				35
2	5	60 min.	x				36
3	2	12 min.		x			37
5	2	60 min.				x	38
5	2	240 min.			x		39

* All exposures were made to water at 49°C.

both of the sites of 9 minute exposures at that temperature show trans-epidermal necrosis. Does this indicate that no epidermal injury had been sustained during the first 7 minutes, or does it mean that injury was present but unrecognizable?

In order to gain more information concerning this point, the experiments summarized in Table V were undertaken. Thermal expo-

sures were made with a running stream of hot water at 49°C. and at atmospheric pressure. Three young pigs were used.

The first series of exposures (reference nos. 1 to 18) were for control purposes and served to establish the reproducibility of reactions to single exposures at this temperature. It may be seen that there was not a single instance in which an exposure for less than 7 minutes caused recognizable necrosis of the epidermis, and that in every instance in which exposures as long as 9 minutes were given there was complete necrosis of the epidermis. Skin sites receiving 7 minute exposures recovered with incomplete or no damage to the epidermis, whereas skin sites receiving 9 minute exposures underwent complete ulceration.

The control exposures were followed by a series (nos. 19 to 39) in which repeated exposures, individually incapable of causing recognizable epidermal injury, were applied to the same area. It was found, for instance, that although a single 3 minute exposure at 49°C. caused no recognizable change in the epithelial cells, three such exposures separated by recovery periods as long as 24 minutes had the same total destructive capacity as a single continuous 9 minute exposure.

It was clear that a certain amount of epidermal injury was sustained during the first 3 minutes and that at least 24 minutes were required before there was an appreciable recovery from this injury. That complete recovery occurred after a period of 2 to 4 hours was indicated by experiments 30 and 31.

Experiments 34 to 39 showed what might have been expected; namely, that recovery from the latent injury of a 2 minute exposure was more rapid and that from a 5 minute exposure less rapid than was the case after a 3 minute exposure.

Further discussion of the implications of these experimental results will be found in Study V of this series.[7]

SUMMARY

The reciprocal relationships of surface temperature and duration of hyperthermia in the production of cutaneous injury have been investigated for pig and man. The data were derived from experiments in which the surface of the skin was brought immediately to, and maintained at, a constant hyperthermic level in such a manner that there was no external mechanical interference with the flow of blood through the skin.

Although there were certain qualitative differences in the reactions of human and porcine skin to excessive heat, there were no significant quantitative differences in their susceptibility to thermal injury in these circumstances.

Time and Temperature in Relation to the Occurrence of Cutaneous Burning

In order to characterize any episode of hyperthermia as critical in respect to its capacity to destroy the epidermis, it is necessary to know both the intensity and the duration of the exposure. When the temperature of the skin is maintained at 44°C., the rate of injurious change exceeds that of recovery by so narrow a margin that an exposure of approximately 6 hours is required before irreversible damage is sustained at the basal cell level. At surface temperatures of 70°C. and higher, the rate of injury so far exceeds that of recovery that less than 1 second is required to cause trans-epidermal necrosis.

At surface temperatures between 44° and 51°C., the total exposure time required to destroy the epidermis is essentially identical to the total duration of the steady thermal state within the epidermis, and, under these circumstances, the rate at which burning occurs is almost doubled with each degree rise in temperature.

Below 44°C. there is a rapid decrease in the rate at which burning occurs and the time-temperature curve is asymptotic in the direction of the time axis. This is probably due to the increased effectiveness of the cellular reparative processes as the hyperthermic level approaches the temperature range that is normal for the tissue.

At surface temperatures greater than 51°C., the exposure time required to destroy the epidermis is so short that during most or all of it the deeper layers of cells are in the process of being brought to, rather than being maintained at, a state of thermal equilibrium with the surface. Thus, as the surface temperature is raised above 51°C., the rate of injury begins to fall off and a time-temperature curve depicting the threshold at which trans-epidermal necrosis occurs is asymptotic in the direction of the temperature axis.

The minimum time required to destroy the epidermis at surface temperatures above 70°C. was not determined. It was observed, however, with exposures at flame temperatures (over 1000°C.), that the amount of time required to raise the temperature at the epidermal-dermal junction to a cell-killing level is so brief that the interposition of anything capable of impeding heat transfer to the skin may be sufficient to make the difference between burning and absence thereof.

Compressive Hyperthermia

Although pressure may increase the rate of heat transfer to the skin, and thereby the rate of burning, by improving the interface contact between it and a solid hot object, there was no evidence that compressive

occlusion of dermal blood vessels has any effect on the susceptibility of the epidermis to thermal injury. When hot water was applied to the surface of the skin at different pressures, it was observed that compressive ischemia did not alter the rate at which burning occurred. It was concluded that the conduction of heat energy away from the skin surface by way of the blood stream does not afford a significant degree of protection against epidermal injury.

Color of Cutaneous Burns

Compression of the skin during exposure to heat may alter the surface color of the resulting burn without affecting its severity. Within a certain range of surface temperature, the application during the exposure of sufficient pressure to blanch the skin may cause a burn to remain ischemic that would otherwise be hyperemic. In such circumstances, differences in color are not indicative of differences in the depth of the injury.

In burns produced without concomitant compression of the skin, the color of the surface of the burn is determined in part by the rapidity and degree of the initial increase in dermal temperature and in part by the duration of the exposure. The surface color of such burns is not a useful criterion for estimating either the severity of injury or the amount of blood that may be pooled in the underlying tissue. When the temperature of the dermis is raised slowly, the superficial vessels become engorged and retain their blood even though the tissue is subsequently coagulated by progressive increase in the intensity of the hyperthermia. When the initial rise in dermal temperature is rapid and high, the superficial vessels contract so quickly that there is no opportunity for them to become hyperemic. Although such burns are superficially ischemic, there is intense hyperemia of the more deeply situated vessels.

REFERENCES

1. Hudack, S., and McMaster, P. D. The gradient of permeability of the skin vessels as influenced by heat, cold, and light. *J. Exper. Med.*, 1932, 55, 431–439. McMaster, P. D., and Hudack, S. II. Induced alterations in the permeability of the lymphatic capillary. *Ibid.*, 1932, 56, 239–253. McMaster, P. D., and Hudack, S. The participation of skin lymphatics in repair of the lesions due to incisions and burns. *Ibid.*, 1934, 60, 479–501.

2. Leach, E. H., Peters, R. A., and Rossiter, R. J. Experimental thermal burns, especially the moderate temperature burn. *Quart. J. Exper. Physiol.*, 1943–44, 32, 67–86.

3. Moritz, A. R. Studies of thermal injury. III. The pathology and pathogenesis of cutaneous burns. An experimental study. *Am. J. Path.* (In press.)

4. Campbell, A. C. P., Alexander, L., and Putnam, T. J. Vascular pattern in various lesions of the human central nervous system; studies with the benzidine stain. *Arch. Neurol. & Psychiat.*, 1938, 39, 1150–1202.

5. Spalteholz, W. Blutgefässe der Haut. Handbuch der Haut- und Geschlechts-krankheiten. J. Springer, Berlin, 1927, 1, Pt. I, 379–433.

6. Moritz, A. R., Henriques, F. C., Jr., Dutra, F. R., and Weisiger, J. R. Studies of thermal injury. IV. Exploration of casualty-producing attributes of conflagrations. The local and systemic effects of generalized cutaneous exposure to excessive circumambient (air) and circumradiant heat of varying duration and intensity. *Arch. Path.*, 1947, 43, 466–488.

7. Henriques, F. C., Jr. Studies of thermal injury. V. The predictability and significance of thermally induced rate processes leading to irreversible epidermal injury. *Arch. Path.*, 1947, 43, 489–502.

8. Henriques, F. C., Jr., and Moritz, A. R. Studies of thermal injury. I. The conduction of heat to and through skin and the temperatures attained therein. A theoretical and experimental investigation. *Am. J. Path.*, 1947, 23, 531–549.

GRANULAR CELL MYOBLASTOMA *

V. R. Khanolkar, M.D.

(From the Tata Memorial Hospital, Bombay, India)

There are several groups of "debatable tumors" whose origin and behavior in the human body are not yet clearly understood. The rarity of some of them has been responsible for lack of accurate definition and separation. Additional information concerning their structure or clinical course is not, therefore, superfluous. The "granular cell myoblastoma" is a tumor of this type. In his original study in 1926 Abrikossoff [1] considered such swellings as degenerative lesions following injury or inflammation. Since then, evidence has been forthcoming which suggests their neoplastic nature and their origin from tissue having competence to develop into striated muscle. In 1931, as a result of a further study, Abrikossoff [2] revised his opinion and suggested that these tumors had their origin in embryonic muscle cells. Gray and Gruenfeld [6] have objected to this interpretation and have stated that "the term myoblastoma should be discarded for this tumor group," as the evidence on which such histogenesis is based is insufficient and the presumed resemblance to embryonal skeletal muscle illusory. These tumors, with rare exceptions, are believed to be stationary or at any rate very slow-growing. They show a predilection for growth in the oropharyngeal region. It has been suggested that they cannot be completely separated from other muscle tumors and that transitions between rhabdomyoblastomas and these tumors are sometimes seen. In a recent study [4] data regarding such tumors from 162 persons have been tabulated and the opinion has been expressed that "definite conclusions as to the histogenesis of the myoblastomas are not warranted at the present time."

During the last 5 years, 10 such tumors were seen at the Tata Memorial Hospital. Only 6 are dealt with here: 2 were encountered in an unusual location, 3 provide interesting information concerning a malignant course in this type of tumor, and one illustrates an interesting histological structure. The remaining 4 resemble closely similar tumors reported in the literature.

REPORT OF CASES

CASE I

A married Sinhalese woman, 32 years old, was referred to the Tata Memorial Hospital (no. 3513) from Colombo, Ceylon, for radiation therapy in December, 1942. She gave a history of having fallen down a flight of five steps in March,

---

1939, landing on her right hip. She suffered from occasional rheumatic pains in the hip, which were relieved by rubbing. In December, 1940, she noticed a small lump (2.5 cm. in diameter) in the right buttock. This gradually increased. The pain in the thigh became more noticeable and ran down the leg into the right calf, but was never severe. She lost about 10 lbs. in weight. In September, 1942, the tumor was ovoid, about 7 by 6 cm. in surface extent, painless, attached to the gluteal muscles, and not fixed to the bone or skin. Roentgenograms did not reveal alteration in the bony structures. A clinical examination failed to show a tumor in the pelvis or elsewhere in the body. The tumor was removed (Dr. Pieris) under general anesthesia. It was embedded in the substance of the gluteus medius muscle and its tendon. It had "the consistency of liver" and was well encapsulated. The surface was not nodular. The wound healed satisfactorily and the patient improved in general health. The tumor was diagnosed as a "malignant endothelioma."

TABLE I
Data Concerning 10 Tumors of Myoblastomatous Nature

Case no.	Location	Age	Sex	Duration
1	Right buttock	32	F	2 years
2	Right buttock	25	M	3 months
3	Left thigh	26	M	3½ months
4	Occipital bone	32	M	6 months
5	Temporal bone	20	M	10 months
6	Middle ear	72	F	1 year
7	Dorsum of tongue	30	F	2 years
8	Left breast	24	F	4 months
9	Breast	31	F	3 years
10	Right loin	42	M	1½ years

A crescentic scar, 25 cm. long, was seen on the outer aspect of the right buttock on examination in December, 1942. The scar was convex downward with its apex about 3 cm. above the great trochanter. There was a moderate loss of substance beneath the scar without evident muscular or sensory disturbance. A little thickening and adherence in the lowest portion of the scar was noticed. At the same time a spherical, hard, movable nodule (2.5 cm. in diameter), which moved with deglutition, was felt in the substance of the thyroid gland. Laboratory investigations were negative. The basal metabolic rate was −11.5 (Mayo normal standard), +1.5 (Indian standard). A piece of the neoplastic tissue was sent to us for histological study and showed the characteristics which will be described later. The right gluteal region was subjected to a total radiation of 1800 r.* through a single portal, 15 by 12 cm., during the course of 1 week. In August, 1943, a hard mass in the right lateral and posterior wall of the pelvic basin was felt during a follow-up examination. The mass was not tender, the general condition of the patient was good, and she had gained 6 lbs. in weight. Further irradiation of 2000 r. was given in 11 days through a 15 by 15 cm. portal on the posterior surface of the buttock. The mass showed no evidence of regression. One month later a small nodule (1.5 cm. in diameter) was felt in the axilla under the lower border of the right pectoralis major muscle. Both breasts were normal. The nodule was excised a fortnight later and showed the same structure as the original gluteal tumor. At that time the lungs were clear and the nodule in the thyroid gland showed no change. The patient returned to her native place in October, 1943, and succumbed a few months later. There was no autopsy.

* Radiation treatment in all cases was administered with a 200 kv. apparatus, under standard conditions of filters and target skin distance.

Gross Examination

The tumor removed at operation was ovoid, distinguishable from the muscle tissue, and well encapsulated. It was firm and on cutting presented a grayish yellow surface broken up into large and small patches by reddish hyaline strands.

Microscopical Examination

The tumor (Figs. 1 to 3) consisted of sheets of closely packed large cells separated by broad bands of pale edematous collagen. The sheets were composed of clusters of cells grouped in a pseudo-alveolar or organoid pattern. The cells in the group lay in close apposition to each other. The clusters were surrounded by a network of slender fibers interspersed with cells having thin, flattened, elongated nuclei. The tumor cells were large (40 to 60 μ), rounded or polyhedral, and contained coarsely granular cytoplasm which was definitely acidophilic. The granules gave the impression of being packed in parallel rows. The nuclei were vesicular, round or ovoid, with a distinct nuclear membrane and scanty, fine, reticulated chromatin. A small round nucleolus was visible in most cells. A few cells showed two or three nuclei. The nuclei were usually centrally placed. No pigment was seen either in the cytoplasm or in the connective tissue cells. Silver impregnation (Gomori) revealed a delicate reticular stroma surrounding individual cells or small groups. The granules did not stain with sudan IV or scharlach R. The tissue was traversed by a rich network of thin-walled blood capillaries; and plugs of tumor cells appeared to lie in venous channels. Unmistakable longitudinal or cross striations could not be seen in the cytoplasm. Mitotic figures were extremely rare.

CASE 2

A young Hindu Surti domestic servant, 25 years old, was referred to the hospital (no. 3714) because of pain and swelling in the right buttock. The pain had started about 3 months previously and extended down the back of the thigh. He had been treated for sciatica by local doctors without relief. He then noticed a swelling in the buttock which gradually increased in size, leading to a limp and later inability to stand or walk. Clinical examination revealed a rounded, firm, elastic mass (27.5 cm. in diameter) extending from the right iliac crest to the corresponding natal fold. Softer areas could be felt in the substance of the tumor. The tumor could be felt deep in the gluteal muscles and was slightly tender. It was not adherent to the skin and did not fluctuate on palpation. A mass was felt also in the right iliac fossa, close to the bone. All thigh movements were restricted and there was a foot drop on the same side. The right hip joint was not affected and the limbs were equal in measurement.

Blood cells were within normal limits. Chemical examination showed blood sugar, 95.2 mg.; phosphorus, 4.4 mg.; calcium, 10.9 mg.; alkaline phosphatase, 3.1 Bodansky units. There was no Bence-Jones protein in the urine. The Kahn test on the blood was negative. Skiagraphic studies showed an invasion and erosion of the pel-

vic bones at the iliopectineal line on the right side. There were no metastases in other bones or in the lungs. A specimen was excised from the tumor in the buttock. It was firm and elastic and had a uniform gray appearance. The tumor was incorrectly diagnosed as lipogenic sarcoma of bone.

The patient was given X-ray irradiation totaling 2000 r. through a 15 cm. circular portal over a period of 3 weeks. The pain was relieved, the patient became afebrile, but the tumor continued to grow in the right iliac fossa. As the condition of the patient deteriorated steadily, he decided to go back to his native place. Owing to difficulties of transportation during war time, he failed to report again.

Microscopical Examination

The section consisted of bundles of tumor cells separated by fibrovascular connective tissue septa. The cells were large and showed forms varying from globular and ovoid, to ribbon-like, long cylindrical cells. The cytoplasm was acidophilic and showed fine and coarse granularity. There was a definite suggestion of longitudinal and transverse striations in some rare cells, particularly near the ends of long cells in the outer cytoplasmic zones. The nuclei were ovoid, more deeply staining and smaller than in case 1, and tended to lie eccentrically in the cell.

CASE 3

A young Englishman, 26 years old (no. 10164), was admitted for X-ray treatment of a swelling in the left groin. He had noticed a lump 3½ months previously. It was not painful but continued to grow. He was treated at another hospital with sulfonamides and, later, with penicillin. The lump gradually increased and the patient became febrile. An enlarged lymph node was removed from the groin and was reported as "Hodgkin's disease" and another piece removed later as "lymphosarcoma, reticulum cell type." When he was seen at the Tata Memorial Hospital in May, 1945, he had a large, globular, soft mass, 15 by 7 by 5 cm., in the left inguinal region. The skin was adherent to the mass in the region of the scars of previous excisions. Another smaller mass was felt in the left iliac fossa. The patient was much emaciated and was maintaining a temperature of about 100°F. He was given about 1300 r. through a 15 cm. portal over the inguinal and iliac regions in a period of 6 days. His condition grew steadily worse; he became drowsy and unconscious. As radiation was having no effect on the tumor mass, the patient was removed to another hospital where he expired 2 days later.

At autopsy, it was found that, besides the large masses in the inguinal and iliac regions, the peritoneum was studded with numerous firm, grayish white nodules. Lymph nodes of the chain from the left iliac region to the para-aortic region were enlarged. The liver and pancreas showed many tumor nodules. There were numerous secondary deposits in both lungs, particularly along the posterior borders. Except for the heart, other viscera, including the cranial contents, were free from neoplastic tissue. The skin and the mucous surfaces were free from tumor, either pigmented or nonpigmented. The eyeballs were not examined. The heart was slightly enlarged and showed numerous grayish white, firm nodules scattered over the surfaces of auricles and ventricles. They were raised very slightly above the general surface. The size of the nodules ranged from 2 to 7 mm. in diameter. In some places

four or five of these had fused together in a larger mass. On opening the heart (Fig. 4) it was seen that the myocardium was sprinkled with tumor nodules, and many were beneath the endocardium. These nodules seemed more numerous on the papillary muscles, giving them a peculiar beaded appearance. Some of the nodules in the liver were much larger and showed central areas of degeneration. The deposits in the lung and pancreas were similar to those described in the heart.

Microscopical Examination

The mass in the inguinal region and iliac fossa as well as the metastatic deposits in the viscera showed a structure similar to that described in cases 1 and 2. In some areas a pseudo-alveolar grouping was noticeable, and in other fields transitions between the globular and ribbonlike cells were evident (Fig. 5). In some of the lung deposits there was a suggestion of striations in the cells.

Comment

Cases 1, 2, and 3 show many similarities in their clinical behavior and histological structure. They have many features in common and bear resemblance to the published descriptions of "granular cell myoblastoma." All of them were malignant in behavior and fatal in their outcome. They showed a pronounced tendency to spread by the lymphatic pathway. The size of the cells, their polymorphism, the presence of intermediate forms between globular and long cylindrical shapes, the character and situation of their nuclei, and the rich and delicate reticulin network around individual cells suggest their affiliation to the striped muscle cells in the body. However, it must be confessed that in the absence of characteristic striations their definite assignment to the group of muscle tumors may not be possible. Doubt may also be entertained that the tumor in case 3 was not a nonpigmented melanoma, but this seems improbable in the light of other findings in this case. These tumors were all insensitive to radiation and one of them continued to grow during treatment.

CASE 4

A Hindu soldier, 32 years old (no. 8494), was admitted because of a swelling in the skull 5 cm. behind the left ear. He had noticed a small lump in that region since childhood. It was quite painless and caused him no inconvenience until about 7 months previous to admission. At that time the swelling had begun to increase rapidly and had become painful and tender. On examination a hard, nodular, irregular, fixed swelling, 12 by 5 cm., was felt in the left parieto-occipital region. The edges of the tumor seemed to merge imperceptibly with the skull. The scalp was freely movable over the tumor, but the growth of hair over it was scanty. The tumor was tender. There were two small nodes slightly to the left of the midline and about 3 cm. below the occipital protuberance.

Laboratory examination showed no abnormality of the cells of the blood, or in its

sugar, phosphorus, calcium and phosphatase content. The Kahn test was negative. Roentgenograms of the skull (Fig. 6) revealed an area of destruction at the parieto-occipital junction, involving the major part of the left half of the suture line and a small portion of the right side. Both tables of the adjoining bones, especially the occipital, were irregularly destroyed. Sclerosis of the upper part of the occipital bone was evident. The rest of the calvarium was normal. No evidence of increased intracranial tension was found. The findings suggested an involvement of the bones by a neoplasm.

Microscopical Examination

The tumor was composed of sheets or groups of cells separated by bands of rich vascular connective tissue. The tumor cells showed marked polymorphism and variation in size. The cells tended to be arranged in parallel ribbons or compact groups. Most of the cells were large and had pale vesicular nuclei. The nucleoli were not prominent. The cell cytoplasm showed fine and coarse granules and was strongly acidophilic. In some places groups of tumor cells were widely separated and tended to be located around blood capillaries. There was an attempt at an organoid pattern in a few areas (Figs. 7 and 8). Remnants of bone spicules were seen embedded in the neoplastic tissue.

CASE 5

A Parsee college student, 20 years old (no. 10470), was admitted for pain and swelling above the left ear. He had noticed slight pain in the region about 10 months previously and a few days later, a tender swelling. He had consulted various practitioners of medicine and finally an otolaryngologist, who treated him by local applications and internal medication. There had been a steady diminution in the acuity of hearing. On examination, a swelling about 5 cm. in diameter was found above and behind the left ear. This had pushed the pinna downward about 1.5 cm. The contour of the skull bulged about 3 cm. at its most prominent point. The swelling was hard, fixed to the skull, and seemed to be arising from the temporal bone. The scalp was freely movable over it. Clinical and laboratory studies showed no other abnormality. There was no evidence of any other tumor. The skiagrams of the skull (Fig. 9) showed extensive destruction of the squama temporalis and posterior portion of the mastoid process, as well as of adjacent portions of the parietal and occipital bones. The petrous portion was not affected.

Microscopical Examination

A portion of the tumor excised for biopsy showed (Fig. 10) groups and rows of polygonal, cylindrical, and fusiform cells in a richly vascular fibrous connective tissue. The cytoplasm of the neoplastic cells was acidophilic and granular. The nuclei were globular or ovoid and showed variation in size, shape, and staining intensity. There was a suggestion of longitudinal striation in some of the cylindrical cells. The diagnosis was granular cell myoblastoma.

The patient was treated with deep X-rays, with a total dose of 3000 r. through a temporal field, 8 by 10 cm., over a period of 3 weeks. There was a slight but definite regression in the size of the swelling. The general condition of the patient

was good. A second skiagram 1 month later showed an upward extension of rarefaction in the squamous bone. Another course of radiation therapy was administered (total, 3000 r. in 3 weeks). At the end of the treatment pain and swelling were very much reduced. At a follow-up examination 2 months later the patient complained of continuous pain in the left external auditory canal. A skiagram showed spread of the disease into the petrous portion of the temporal bone with increased destruction of bony tissue, but with foci of early sclerosis in the squamous portion of the temporal bone. He was readmitted for treatment 3 months later with unbearable deep pain in the ear. The skiagram showed extensive spread of the disease in the parietal bone.

Comment

In the available literature I have not encountered reports of tumors in the skull such as were found in cases 4 and 5. They have been reported as occurring in facial bones, either the maxilla or the mandible, and mostly in infants at birth. A majority of them were recorded as congenital epulides of the newborn.[4] Tumors of the external auditory canal will be referred to later. The 2 cases reported here afforded interesting material for speculation regarding their histogenesis.

CASE 6

A Parsee woman, 72 years old (no. 14231), was seen with a complaint of pain in the left ear. She gave a history of intermittent purulent discharge from both ears since early childhood. The present difficulty had started about 1 year previously with complete deafness in the left ear. She noticed a little plug of tissue in her outer ear which bled occasionally. Her condition was diagnosed as "polyps" and she was treated with ear drops and repeated removal of projecting bits of tissue, once or twice a week for about 9 months. During this period her pain became worse, and, because it persisted, she saw an otologist who removed the polypoid tissue in the ear and had the pieces examined histologically. They were reported as "fibrous clot in a stage of partial organization." The specialist observed that "the growth was coming from the middle ear, probably the medial wall, but the exact site was impossible to determine; bleeding was not excessive." The patient felt much better after the operation and was relieved of pain and bleeding. A fresh lump began to protrude from the outer meatus within a fortnight. On examination at this hospital it was found that the external auditory meatus was completely filled with a soft grayish white tissue. At the time of examination here she complained of fever, of an obstruction in the left nostril, and of a slight pain on swallowing on that side. A smooth, soft, glandular, polypoid swelling could be seen in the region of the pharyngeal opening of the eustachian tube. It bled easily when touched with a gloved finger. A specimen taken for biopsy from the tumor mass in the external auditory meatus showed the characteristics described below. At operation, as much of the tumor as could be seen was removed from the external auditory meatus. The patient felt much better and her temperature decreased. The tumor mass in the nasopharynx was not altered; the patient is still under observation.

Microscopical Examination

The tumor was composed in its deeper parts of sheets of closely packed, large, pale cells with abundant granular acidophilic cytoplasm. The cells were spheroidal or elongated. The nuclei were relatively small and centrally placed in most cells. There were, however, several

cells showing two or three nuclei huddled together. The cells were held in a delicate reticular and fibrous network with slender capillaries. Mitotic figures were frequent. Some cells showed a suggestion of longitudinal striation, but definite cross or longitudinal striation was not found. Lipoid material could not be demonstrated in the cytoplasm with scharlach R. In the superficial areas there was secondary inflammation with surface necrosis and richly vascular granulation tissue interspersed with isolated tumor cells (Fig. 12).

Comment

Granular cell myoblastomas in the region of the outer ear have been described by Horn and Stout [8] and by Altmann [3] as smooth-lobed or pedunculated, filling the external auditory canal and growing slowly. They attracted attention by bleeding from the ear. A gradual extension involved neighboring structures later in the course of the disease. The case reported here showed extension outward from the tympanic cavity and inward through the eustachian tube. The cells comprising the tumor were more isolated, rounder, and smaller than the benign type of granular cell myoblastoma. In many areas their characteristics were suggestive of a malignant neoplasm and a diagnosis of polymorphous cell sarcoma could not be ruled out definitely.

Discussion

A perusal of the literature on myoblastomas and a study of the pathological material available here have raised questions which may be worth considering.

1. The nature of these tumors, whether they are degenerative processes, productive inflammatory lesions, or true neoplasms, has engaged the attention of many observers. It has even been suggested that tumors of this type are histogenetically dissimilar in different locations and that the myoblastomas of the tongue are unlike those occurring in other regions. These varying opinions lose much of their subtlety when it is realized that in many tumors, "however closely the processes are analyzed, the conclusion remains that inflammatory hyperplasia passes into neoplasia." [5] The close resemblance between the cellular constituents of these tumors and the forms encountered during regeneration of voluntary muscle does not necessarily imply an absence of neoplastic growth but may indicate the various phases during the course of a differentiation of tumor cells. The regenerating tissue following injury or inflammation is not as susceptible as healthy tissue to stimuli which are responsible for maintaining the normal pattern of the organism. It

should therefore be expected that processes leading to repeated destruction and regeneration would also supply the conditions necessary for neoplastic growth. During the past 100 years, pathologists have been obsessed with the idea that neoplasia is an entirely unnatural and abnormal process and not an outcome of interdependent biological events. "When tumor formation is conceived as a necessary, innate and therefore physiological reaction induced by one or other of unnumbered, interchangeable stimuli, and when it is interpreted as resumed if 'pathological' *development,* the mystery commonly said to obscure it is seen to be 'entirely owing to ourselves'." [11] The features which usually characterize a neoplastic growth are that it is progressive, expansive, and infiltrative. In the examples which have been reported above, all of these characters were discernible and there could be no uncertainty regarding their neoplastic nature.

2. The second question concerns their designation. The gross and microscopical characteristics assigned to the group of tumors termed "granular cell myoblastoma" are as follows: They are usually small, circumscribed, encapsulated, spherical, and lobulated masses. Their consistency is firm. On section they present a grayish yellow or tan surface. In some tumors the borders are indistinct and the neoplastic tissue appears to penetrate irregularly in the adjoining muscle. Histological examination shows the tumor to be composed of rather large polyhedral cells, with abundant acidophilic cytoplasm separated in clusters, bundles, or sheets by wide strands of edematous connective tissue. The large size of the cells (50 to 60 μ in diameter) and the coarse or fine refractive granules in the cytoplasm constitute arresting features. The reticulum forms a delicate network between and around individual cells or small groups of cells. The cytoplasm appears red with Masson's trichrome stain and brownish yellow with van Gieson's stain. The granules do not possess the tinctorial properties of glycogen or lipoid. The nuclei are usually small and vesicular, with distinct nuclear membrane and an inconspicuous karyosome. These tumors possess other microscopical characteristics which are interesting. The tumor cells exhibit marked polymorphism with globular cells at one extreme and ribbon-like, long cylindrical cells at the other. Intermediate forms described as teardrop, tadpole, and banjo shapes as well as irregular syncytial masses are encountered. In some tumors there is a marked tendency toward a grouping of cells into pseudo-acinar clusters. These clusters, however, do not show the sharply defined lumina which characterize glandular acini. The presence of longitudinal and cross striations in the cytoplasm of these tumors has given rise to much

discussion. Microscopical appearances highly suggestive of such striations sometimes are met with in the peripheral zones of cells in suitably fixed material.

All of the tumors referred to in Table I satisfy most of the microscopical criteria mentioned above. The histological characteristics as well as the gross appearance of the tumor as it is seen embedded in the substance of a muscle are highly suggestive but not conclusive evidences of its origin from muscle cells. Tissue culture studies have not established the myoblastic nature of these cells. In a personal communication,[13] Dr. A. P. Stout has stated: "Dr. Margaret Murray has grown some of these tumors *in vitro*. They do not behave altogether like striated muscle cells, either benign or malignant. The granular cells wander out and then lose their granules, nor do they reappear again when the cells reproduce." The main difficulty remains as to their relationship, if any, to the rhabdomyomas and rhabdomyosarcomas. A separation of granular cell myoblastomas from myosarcomas appears justifiable in view of a difference in their clinical behavior, which will be referred to later. Abrikossoff[2] (1931) separated a variety with polymorphous cells and areas of frankly sarcomatous nature as his fourth group of myoblastic myomas. Stout[8] has rightly pointed out that this fourth group is really rhabdomyosarcoma and not at all like the first three, because in it the cells are not granular, but resemble those of polymorphous cell sarcomas. He is of the opinion that Abrikossoff had no justification for including the fourth group with the first three.

3. Granular cell myoblastomas have been described as slow-growing benign tumors which do not usually recur after adequate resection. Other observers have found that the incidence of malignancy of "this ordinarily benign tumor cannot at best exceed 10 per cent and is probably not as high as this."[13] Ravich, Stout, and Ravich,[12] while describing their only case of malignant granular cell myoblastoma, emphasized the important histological and clinical distinctions between the benign tumors described by Abrikossoff[2] and the fourth malignant group. They stated that "until the case here reported was observed by us we were firmly convinced that no example of a malignant primary granular cell myoblastoma belonging to any of Abrikossoff's first three groups had ever been recorded." They were satisfied that neither the 5 cases of malignant myoblastoma described by Howe and Warren[9] nor the other 10 culled by them from the literature belonged to Abrikossoff's first three groups in their primary manifestations. In view of this opinion I have pondered over the cases described in this paper and have hesitated before presenting them for fear of recording doubtful

instances of metastasizing granular cell myoblastomas. Repeated study of these cases, however, leaves no other option than to classify them as examples of the third group of tumors described by Abrikossoff. The tumors in the first two patients presented a relatively benign appearance in histological preparations. The cells were large and polygonal, and contained faintly oxyphilic granules which did not show the staining characteristics of lipoid. Case 6 shows many interesting features. Clinically, the tumor was diagnosed for many months as a slow-growing benign papilloma. Microscopic examination showed features associated with active growth and consisted of cells ranging from the large polygonal type, with well marked acidophilic granular cytoplasm, to polymorphous cells showing marked anaplasia, deep-staining nuclei, faintly basophilic homogeneous cytoplasm, and several mitotic figures. The histological features are not so characteristic in cases 3 and 5 and it is possible that the error of mistaking a polymorphous cell sarcoma for a malignant form of myoblastoma may have been committed. It may, however, be pointed out that the histological picture closely approximates that shown by Figure 3 of the published article of Horn and Stout [8] and the description accompanying their case 2.

The essential criteria for a malignant tumor have been accepted as some or all of the following: (1) Hyperplasia of cells beyond that ordinarily seen in an inflammatory process; (2) an atypical quality of tumor cells—anaplasia; and (3) displacement or transportation of tumor cells from the place of their origin. The first three tumors reported here, besides exhibiting the microscopic features of a granular cell myoblastoma, showed evidence of a progressive growth with a spread along the lymph channels and a clinically malignant course. It is necessary to admit that no reasonable explanation can be offered for the unduly large number of cases in this series showing malignant evolution, and it is for this very reason that I had the temerity to add to the literature on the subject. It may be suggested that, though a majority of these tumors evolve slowly and show a benign course, others may grow rapidly, spread along lymphatics, and give rise to wide dissemination with a fatal termination. Experimental work on cancer has tended to establish that "Malignancy is a universal cell potentiality in that any cell has inherent in its makeup the potentiality for unlimited or uncontrolled growth." [10] The differences of opinion concern the relative incidence of malignancy in tumors of this type and whether the malignant tumors should be grouped with polymorphous cell sarcomas, rhabdomyosarcomas, or the malignant variety of granular cell myoblastoma. The competence of cells to undergo malignant proliferation is a property which cannot necessarily be correlated with

their histological features. This was particularly noticeable in some of the cases described above, as it was bewildering to follow a relentless clinical course coupled with a relatively innocent histological appearance. "No morphologic terms and characteristics can serve to indicate the developmental potencies of cells." [7]

4. The occurrence of granular myoblastomas in localities where striped muscle is not normally found has been explained on the basis of a close embryologic association of the precursors of the skin, the muscle and the bone; or a displaced persistence of embryonic cell rests. The work of experimental morphologists makes it unnecessary to seek for such cell rests in the genesis of most neoplasms. There is accumulating evidence to show that birth does not abolish the developmental potentialities of cells along certain specific directions. The mesenchymal cells in the vicinity of small blood vessels are "endowed with all potencies of embryonic mesenchyme." It is conceivable that mesenchymal cells may take on a neoplastic growth under appropriate stimuli and that the cells may assume a structure along the direction of their formative potencies. The structure of the resulting new growth may not necessarily resemble closely the structure and arrangement of cells possessing such potencies. It therefore seems unprofitable to assume the existence of embryonic cell rests at the sites of origin of the last three tumors (cases 4, 5, and 6).

REFERENCES

A comprehensive bibliography has been given by Crane and Tremblay[4] (1945). Therefore, only a few references need be cited.

1. Abrikossoff, A. Über Myome, ausgehend von der quergestreiften willkürlichen Muskulatur. *Virchows Arch. f. path. Anat.*, 1926, 260, 215–233.

2. Abrikossoff, A. I. Weitere Untersuchungen über Myoblastenmyome. *Virchows Arch. f. path. Anat.*, 1931, 280, 723–740.

3. Altmann, F. Granular cell myoblastomas of external auditory meatus. *Laryngoscope*, 1943, 53, 195–202.

4. Crane, A. R., and Tremblay, R. G. Myoblastoma (granular cell myoblastoma or myoblastic myoma). *Am. J. Path.*, 1945, 21, 357–375.

5. Ewing, J. Neoplastic Diseases. W. B. Saunders Co., Philadelphia, 1940, ed. 4, pp. 247–248.

6. Gray, S. H., and Gruenfeld, G. E. Myoblastoma. *Am. J. Cancer*, 1937, 30, 699–708.

7. Gruenwald, P. A note on the so-called undifferentiated and embryonic cells. *Arch. Path.*, 1943, 36, 190–194.

8. Horn, R. C., Jr., and Stout, A. P. Granular cell myoblastoma. *Surg., Gynec. & Obst.,* 1943, 76, 315–318.

9. Howe, C. W., and Warren, S. Myoblastoma. *Surgery,* 1944, 16, 319–347.

10. Murphy, J. B. An analysis of the trends in cancer research. *J. A. M. A.,* 1942, 120, 107–111.

11. Nicholson, G. W. Studies on tumour formation. XX. Induction and determination. *Guy's Hosp. Rep.,* 1938, 88, 263–298.

12. Ravich, A., Stout, A. P., and Ravich, R. A. Malignant granular cell myoblastoma involving the urinary bladder. *Ann. Surg.,* 1945, 121, 361–372.

13. Stout, A. P. Personal communication.

[Illustrations follow]

DESCRIPTION OF PLATES

PLATE 117

Fig. 1. Case 1. Sheets of large polyhedral cells are interspersed with thin-walled blood capillaries. Hematoxylin and eosin stain. × 170.

Fig. 2. Case 1. Three large polyhedral cells with characteristic granules are shown at higher magnification. Masson's trichrome stain. × 750.

Fig. 3. Case 1. A delicate reticular stroma surrounds individual cells and small groups. Gomori's silver impregnation stain. × 170.

Fig. 4. Case 3. Transverse section of the heart, showing the inferior half. The tumor nodules in the ventricular walls and the papillary muscles are clearly seen.

Khanolkar

Granular Cell Myoblastoma

PLATE 118

FIG. 5. Case 3. Tumor deposits in the heart muscle. The cardiac muscle fibers are seen as a darker band between two deposits. Hematoxylin and eosin stain. × 130.

FIG. 6. Case 4. Lateral view of the skull, showing an area of destruction at the parieto-occipital junction.

FIG. 7. Case 4. The character of the granular cells, the details of the nuclear structure, and the intercellular reticulum are shown. Masson's trichrome stain. × 750.

FIG. 8. Case 4. Sheets of tumor cells with an attempt at an organoid pattern. Hematoxylin and eosin stain. × 170.

PLATE 118

5

7

8

6

Granular Cell Myoblastoma

Khanolkar

PLATE 119

Granular Cell Myoblastoma

Khanolkar

SUBCORTICAL FIBROBLASTOMA OF THE BRAIN

A Case Report *

Desiderius Groszberg, M.D., and Irving J. Blumenthal, M.D.

(From the Neuropsychiatric Research Unit, Veterans Administration Hospital, Northport, L.I., New York, and the Veterans Administration Hospital, Bedford, Mass.)

Before describing a case of subcortical fibroblastoma of the brain, it is thought advisable to summarize the numerous and often conflicting views pertaining to the origin of such tumors. Different authors have formulated different theories, or have demonstrated seemingly convincing proofs, relating to the derivation of certain tumors of the central nervous system. Each has proposed what seemed to him to be the most appropriate name to characterize adequately the nature of the growth. The term dural endothelioma was frequently employed until Mallory[1] showed that the type cell of these tumors was the fibroblast of the arachnoid membrane. It logically followed that they received the name arachnoidal fibroblastoma. Cushing,[2] however, preferred the more inclusive term meningioma, which Learmonth[3] modified to leptomeningioma, thereby excluding the possible suggestion of dural origin. The name meningeal fibroblastoma advanced by Penfield[4] is, however, more of a blanket expression than Mallory's. In one article, Alpers, Yaskin, and Grant[5] use only the simple fibroblastoma designation, while in another report,[6] in which 75 such tumors are analyzed from several standpoints, the term meningeal fibroblastoma is employed. In reporting one of Mallory's specimens, Bailey[7] also called it simply fibroblastoma. In order to express the origin of the growth, Elsberg[8] preferred Penfield's designation of meningeal fibroblastoma.

Globus[9] classified the meningiomas from phylogenetic and ontogenetic points of view, and emphasized that fibroblastoma is not descriptive of the majority of meningiomas and that it can be applied only to a small subgroup. He proposed subdivision into five types based on origin and structure: (1) Meningioma indifferentiale, mesenchymatous meningioma; (2) Meningioma omniforme, primitive meningioma; (3) Pachymeningioma, fibroblastic meningioma or dural fibroblastoma; (4) Leptomeningioma, arachnoid (?) meningioma; (5) Meningioma piale, pial (vascular) meningioma. He conceded, however, that "the fibroblastic character is not denied for many of the cellular elements in a large number of meningeal tumors nor for the dominant cell type of a few."

As long as there is general agreement among all the authors that these tumors arise in the meninges, whether on the surface or in the prolongations carried by the blood vessels into the deeper layers of the parenchyma, it seems that the nature of the growth is adequately characterized by the designation of the cell type of which it is composed, regardless of the original location of the cell, provided that this is accepted as a fibroblast. The difference of opinion, indeed, pertains rather to the type of meningeal membrane than to the type of cell.

While many fibroblastomas with or without qualifying adjectives have been reported in the literature, most of those tumors were surface growths. That is, although they pressed deeply into the brain substance, they were at one place attached to the overlying dura. According to Babcock,[10] "Meningiomas constitute one-eighth of all brain tumors." Elsberg[8] stated that "probably 90 per cent of meningeal growths are fibroblastomas." Kazan, Weller, and Jaramillo[11] observed that "it is commonly stated that meningiomas comprise some 15 per cent of all forms of brain tumor" and that of 431 cases of brain tumors found at autopsy, 74 (17.1 per cent) were meningiomas.

Up to 1932, according to Alpers, Yaskin, and Grant,[5] only three fibroblastomas were known to have been reported that were subcortical and without surface connection. However, another deep intracerebral fibroblastoma was described by Petit-Dutaillis and Bertrand,[12] and Baker and Adams[13] reported a subcortical tumor in the right middle and inferior frontal convolutions which, although not encapsulated, was well demarcated from the surrounding brain tissue. Histologically, it consisted of numerous fine and coarse strands of intertwining collagenous fibers with a moderate number of cells interspersed. The authors identified the cells as fibroblasts and called the tumor a fibroblastoma. These five so-called intracerebral fibroblastomas were in the brain substance. Similar growths of another variety which are intraventricular and originate probably in the choroid villi are not included in this group. Such tumors were described by Gardner and Turner[14] who give the pertinent references.

As a closely related finding, a case of subcortical meningioma of the cerebellum was reported by Christophe and Divry,[15] who quoted the histological description of the growth by Harvey Cushing, whose opinion they had obtained: "Case M. . . . proves to be a typical meningioma and although intracerebellar subcortical meningiomas are so rare that we have no definite example in our series of 315 cases, nevertheless there is no reason why it cannot occur. Just where it has taken its origin is difficult to surmise, but I would suppose that it might have come off from the chorioid plexus or from the tela chorioidea superior

(velum interpositum) which have been described as [sources of] subcortical meningiomas of cerebral hemispheres without dural attachments."

In an attempt to differentiate these tumors from those that invade the brain substance from its surface, they were named primary fibroblastomas. This should not be misinterpreted, however, to mean that such a tumor is a first focus that may give rise to secondary growths elsewhere. These tumors *do not* metastasize. Indeed, one of their chief characteristics is that they are completely encapsulated and more or less easily shelled out from their nests; and, in spite of a microscopical structure that strongly resembles fibrosarcoma, they are classified as nonmalignant tumors. From the clinical viewpoint, because of their location and size, these neoplasms produce severe symptoms, depending upon the nature of the brain tissue which is affected. Many were causes of death, directly or indirectly.

REPORT OF CASE

The record accompanying the part of a tumor that was sent to this Unit by the Veterans Administration Hospital, Bedford, Massachusetts, may be abstracted as follows:

The patient was a male, 54 years old, who was admitted on July 6, 1945, and was diagnosed as having a psychosis with syphilitic meningo-encephalitis, and right hemiparesis, on August 24, 1945. A metastatic carcinoma was considered upon admission, apparently because of the right hemiplegia and loss of weight. There had been hemorrhage from the stomach and melena in April, 1944. Because of his mental state, which was marked chiefly by loss of appetite, inaccessibility, and broodiness, this patient had received seven electroshock treatments with temporary benefit.

The patient had gross hematuria on July 14, 1945. The spinal fluid on July 17, 1945, was clear; globulin, large amount; cells, 6; total protein, 150 mg.; Wassermann reaction, suggestively positive in 0.5 cc., positive in 1.0 cc.; colloidal gold test, 4433321000.

On October 8, 1945, the patient had a temperature of 103°F. The clinical impression was that of advanced carcinoma. He received penicillin, as well as soluble vitamin B, twice daily. On November 2, 1945, there was edema of the right forearm and hand, with blebs; pulsations in the arm were good. The patient expired the next day.

Clinical Diagnoses. Right hemiparesis, possible peptic ulcer, decubital ulcer over sacrum.

AUTOPSY FINDINGS

The post-mortem examination (I. J. B.) was performed at the Veterans Administration Hospital, Bedford, Massachusetts. The body was markedly undernourished. The right forearm showed denuding of the skin in large patches, approximately the size of the palm of the hand. The skin was bluish. There was a sharp line of demarcation approximately 1 inch above the cubital fossa. The right hand, itself, was swol-

len. There were several blisters upon the back of the right hand, the largest approximately 1 inch in diameter. Within the blisters, a clear fluid was found. Dissection of the cubital fossa disclosed that practically all of the veins were occluded. The thrombotic material appeared to be very fresh. Within the tissue a serous fluid was found. The arteries appeared normal. A healing excoriation was found at the right elbow, upon the dorsal surface. The subcutaneous tissues appeared to be edematous in this region. No evidence of gas formation was found within the tissues themselves.

There was a large decubital ulcer at the base of the spine, measuring approximately 4 by 3 inches. The subcutaneous tissue in this area appeared somewhat necrotic. Several ulcerations of the posterior surface of the right lower extremity were found also. One measured approximately 4 inches, the other approximately 3 inches in length, and each approximately 1 inch in width.

Head. Scalp and skull appeared normal. The surface of the brain showed no abnormality. Section of the brain disclosed a tumorous mass, 2½ by 1¾ by 1½ inches, in the left frontal lobe. This mass was rather firm and pale pink. It appeared to be encapsulated and shelled easily out of the adjacent brain tissue. It also appeared to be somewhat lobulated. The rest of the brain showed no abnormality. The basal vessels showed no arteriosclerosis. The base of the skull appeared normal. The brain itself weighed 1420 gm.

Thorax. The left lung weighed 390, the right, 570 gm. The left lung showed adhesions to both the lateral and posterior walls of the chest. However, on section both lungs appeared normal. The heart was normal in size and shape and its musculature and valves were normal. The aorta showed no lesions. The coronaries were patent and normal throughout.

Abdomen. The intestines appeared normal, as did the stomach and pancreas. The liver was normal in size and shape. The adrenals were normal. The left kidney weighed 150, the right, 110 gm. The capsules stripped with ease. Sections of both kidneys were normal. The abdominal aorta was normal grossly. The spleen weighed 100 gm. and its surface was slate-colored. The section was dark red and fairly firm, with many whitish strands strewn throughout the splenic pulp. The gallbladder showed no abnormality.

Male Pelvis. No abnormal findings were noted in the pelvis.

Pathological Diagnoses (Including Histological Findings). Tumor of brain, left frontal lobe (meningeal fibroma); thrombophlebitis, with moist gangrene, right forearm; adhesive pleurisy, left lung.

Gross Description *

The specimen was a grayish white lump of tissue, one side of which was flat where it had been cut before it was sent to this laboratory. The cut surface was roughly triangular with rounded corners. Opposite the cut surface the tissue was rounded, somewhat nodular, and encased in a thin capsule. At one side of the triangular surface there were fragments of connective tissue bands which gave the impression that it was there that the tumor had been attached to the surrounding structures and had been severed surgically. The dimensions were approximately 32 by 25 by 22 mm. for the three main diameters. The nodule had a firm consistency, with the elasticity of a medium-hard rubber eraser. The cut surface showed very light gray granular areas which were confluent and occupied the greater part of the cut surface. Between these, the tissue appeared firmer and more like connective tissue, and here there were many small, dark spots ranging in size from that of a pin-point to that of a millet seed, evidently transverse sections of blood vessels or minute hemorrhages.

Microscopical Description *

Some of the sections were stained with hematoxylin and eosin, others were impregnated with silver by Perdrau's method.

There was a strong capsule, consisting mainly of connective tissue fibers of collagenous character. Branches of the connective tissue bundles formed a dense network with an abundance of the cellular elements. The cells of the matrix varied in size, shape, and structure. Some had round or oval, large, lightly stained vesicular nuclei in which there was a fine keratin network, at the intersections of which there were small chromatin granules. Other cells had small, darkly stained nuclei. The cytoplasm was usually elongated, fusiform, and stained fairly homogeneously with eosin. From the periphery both fibrous and cellular elements proceeded into the deeper layers. The cells formed dense, solid cords and masses which spread in an irregular pattern in all directions. While they were richly cellular, no purposeful arrangement or attempt to form some differentiated structure could be observed. Indeed, the polarity of the cells was completely upset. In some areas there were massive cell groups without discernible connective tissue fibers, strongly resembling malignant growths of mesodermal origin. Disseminated among the other cells were many giant cells with several nuclei which were closely packed at the central portion of the cytoplasm. The cells forming the cords and masses varied from large

---

oval elements with large vesicular nuclei, to small cells with dark pyknotic nuclei.

There were several areas in which the cells assumed a spiral arrangement around either a small blood vessel or a homogeneously stained acidophilic lump which seemed to be composed of coarse bands resembling a loose skein. Some of these cell groups formed conspicuous whorls of various sizes. The tendency of the cell masses to encircle foci of other elements was very prevalent.

Perdrau's silver impregnation method brought out large bundles of collagenous fibers composed of coarse and fine strands, as well as very delicate reticulin, the filaments of which formed a complex network. The fibers surrounded the blood vessels in a massive, dense skein and then branched off into a gradually looser network. The capsule itself was composed of fibrous elements of the same type.

The whole tissue was richly supplied with blood vessels ranging from approximately 500 μ in diameter to a network of dense capillaries. All blood vessels were filled to capacity with red blood cells. There were several whose walls showed evidence of a break-through by adjacent tumor masses. There, the invading cells could be seen in the lumina.

While no definitely characteristic mitotic figures were identified, there were many extremely dense, darkly stained elements which perhaps represented phases of rapid cell division.

Against the microscopical structure, which is strongly suggestive of a malignant tumor, stand the gross findings of a growth which was encapsulated and easily separated from the adjacent brain substance. Considering both the gross appearance and the microscopical structure, a diagnosis was made of subcortical fibroblastoma of the brain.

SUMMARY

1. Dural endothelioma, arachnoidal fibroblastoma, meningioma, leptomeningioma, meningeal fibroblastoma, and fibroblastoma of the brain are synonymous terms used by different authors to designate the same tumor which is comparatively common as a surface growth.

2. The so-called primary or subcortical variety of this neoplasm is very rare. An example of subcortical fibroblastoma, which was found at autopsy, is the basis for this report.

REFERENCES

1. Mallory, F. B. The type cell of the so-called dural endothelioma. *J. M. Research*, 1919–20, 41, 349–364.
2. Cushing, H. The meningiomas (dural endotheliomas): their source, and favoured seats of origin. *Brain*, 1922, 45, 282–316.
3. Learmonth, J. R. On leptomeningiomas (endotheliomas) of the spinal cord. *Brit. J. Surg.*, 1926–27, 14, 397–471.

4. Penfield, W. The encapsulated tumors of the nervous system. Meningeal fibroblastomata, perineurial fibroblastomata and neurofibromata of von Recklinghausen. *Surg., Gynec. & Obst.,* 1927, 45, 178–188.

5. Alpers, B. J., Yaskin, J. C., and Grant, F. C. Primary fibroblastoma of the brain. *Arch. Neurol. & Psychiat.,* 1932, 27, 270–281.

6. Frazier, C. H., and Alpers, B. J. Meningeal fibroblastomas of the cerebrum; a clinicopathologic analysis of 75 cases. *Arch. Neurol. & Psychiat.,* 1933, 29, 935–989.

7. Bailey, P. Intracranial sarcomatous tumors of leptomeningeal origin. *Arch. Surg.,* 1929, 18, 1359–1402.

8. Elsberg, C. A. The parasagittal meningeal fibroblastomas. *Bull. Neurol. Inst. New York,* 1931, 1, 389–418.

9. Globus, J. H. Meningiomas; origin, divergence in structure and relationship to contiguous tissues in light of phylogenesis and ontogenesis of the meninges, with suggestion of a simplified classification of meningeal neoplasms. *Proc. A. Research Nerv. & Ment. Dis.,* 1937, 16, 210–265. (Also: *Arch. Neurol. & Psychiat.,* 1937, 38, 667–712.)

10. Babcock, W. W. Large encapsulated meningioma invading the parietal lobe of the brain. *S. Clin. North America,* 1928, 8, 769–772.

11. Kazan, A. T., Weller, D., and Jaramillo, J. G. Intracranial meningiomas. *J. Mt. Sinai Hosp.,* 1944, 11, 105–128; 169–184.

12. Petit-Dutaillis, D., and Bertrand, I. Fibroblastome profond, intracérébral de l'hémisphère gauche, sans connexions méningées décelables. Ablation de la tumeur. Guérison opératoire. *Rev. neurol.,* 1932, 2, 96–100.

13. Baker, A. B., and Adams, J. M. Primary fibroblastoma of the brain. Report of a case. *Am. J. Path.,* 1937, 13, 129–137.

14. Gardner, W. J., and Turner, O. A. Primary fibroblastic tumors of the choroid plexus of the lateral ventricles; a clinicopathological study of three cases. *Surg., Gynec. & Obst.,* 1938, 66, 804–809.

15. Christophe, L., and Divry, P. Méningiome intracérébelleux. *J. belge de neurol. et de psychiat.,* 1937, 37, 575–582.

[Illustrations follow]

DESCRIPTION OF PLATES

PLATE 120

FIG. 1. Cellular and fibrous elements in approximately equal proportions. Giant cells. Hematoxylin and eosin stain. × 100.

FIG. 2. Cellular area showing a great variety of nuclei and masses without formation of differentiated structures. Hematoxylin and eosin stain. × 100.

Groszberg and Blumenthal   Subcortical Fibroblastoma of the Brain
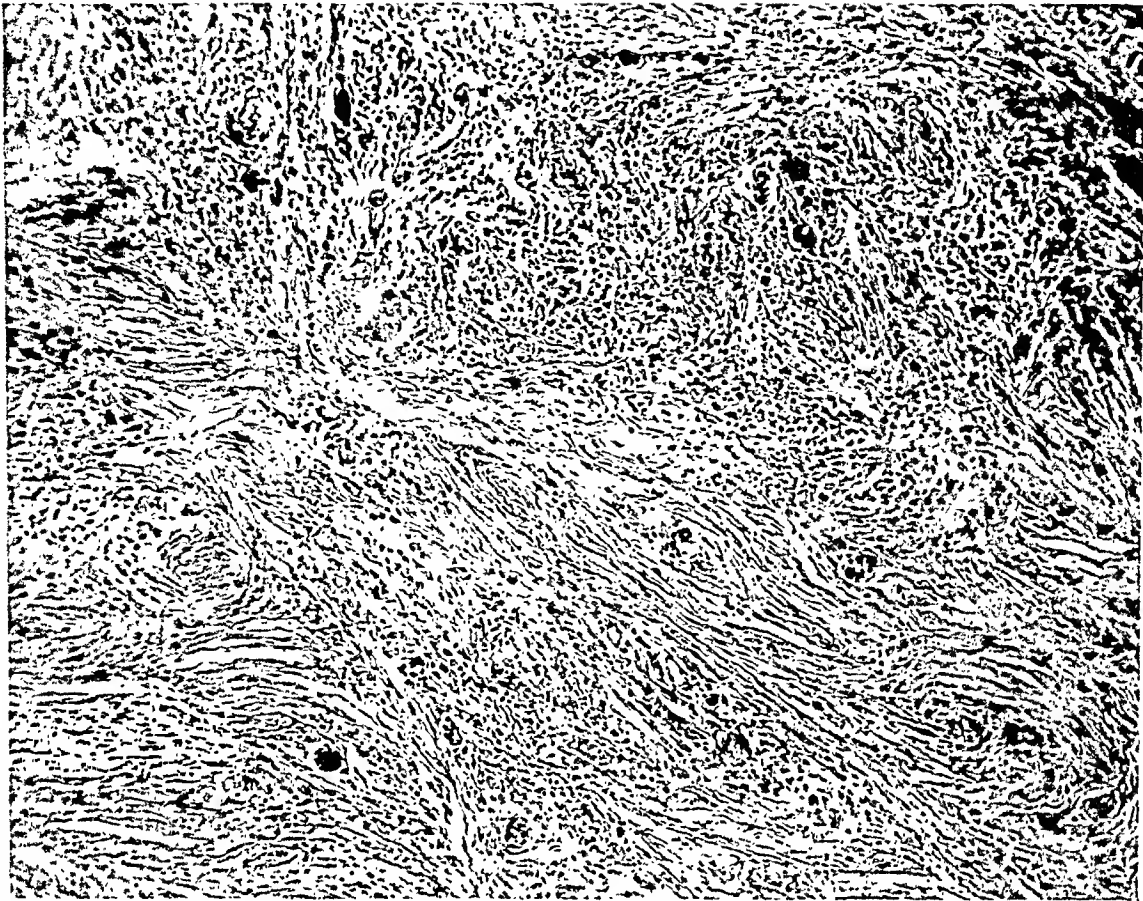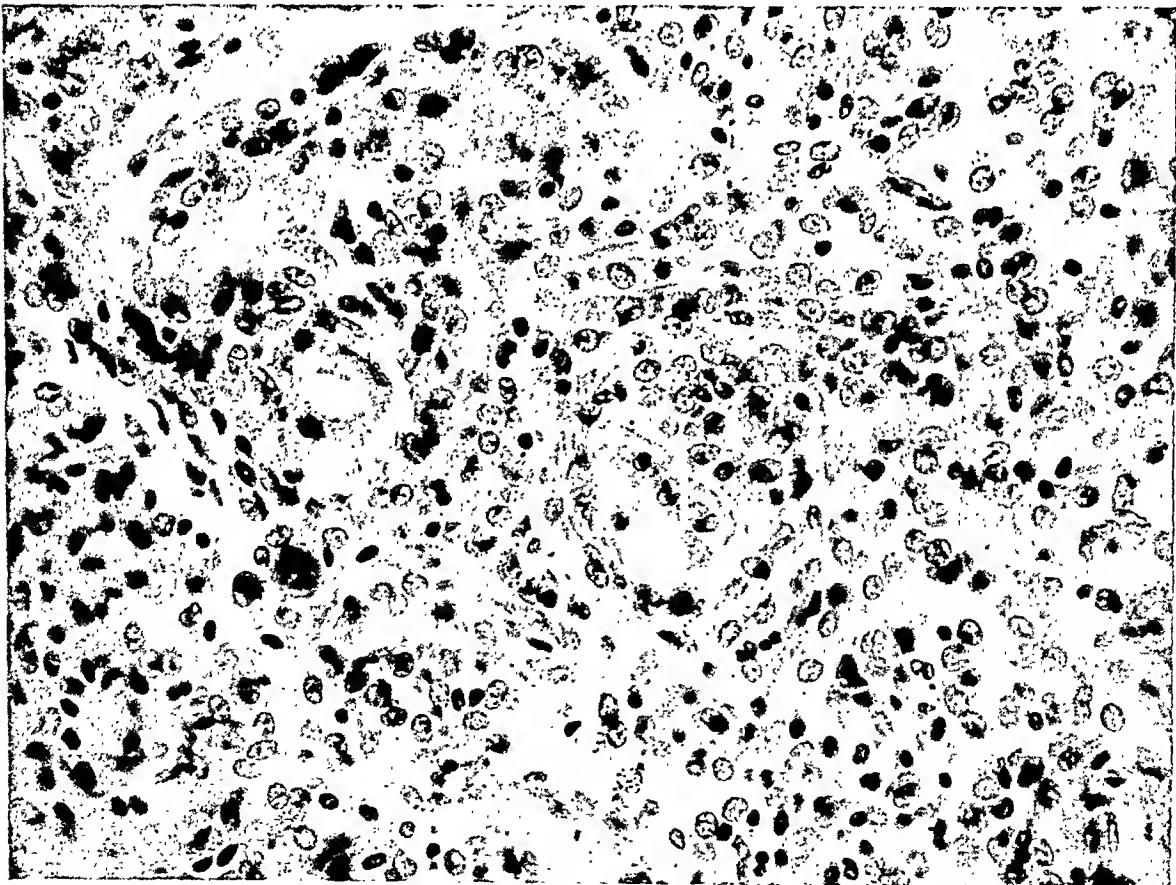
FIG. 3. Whorl formation. Hematoxylin and eosin stain. × 100.

FIG. 4. The center whorl of Figure 3. Hematoxylin and eosin stain. × 430.

3

4

Groszberg and Blumenthal   Subcortical Fibroblastoma of the Brain
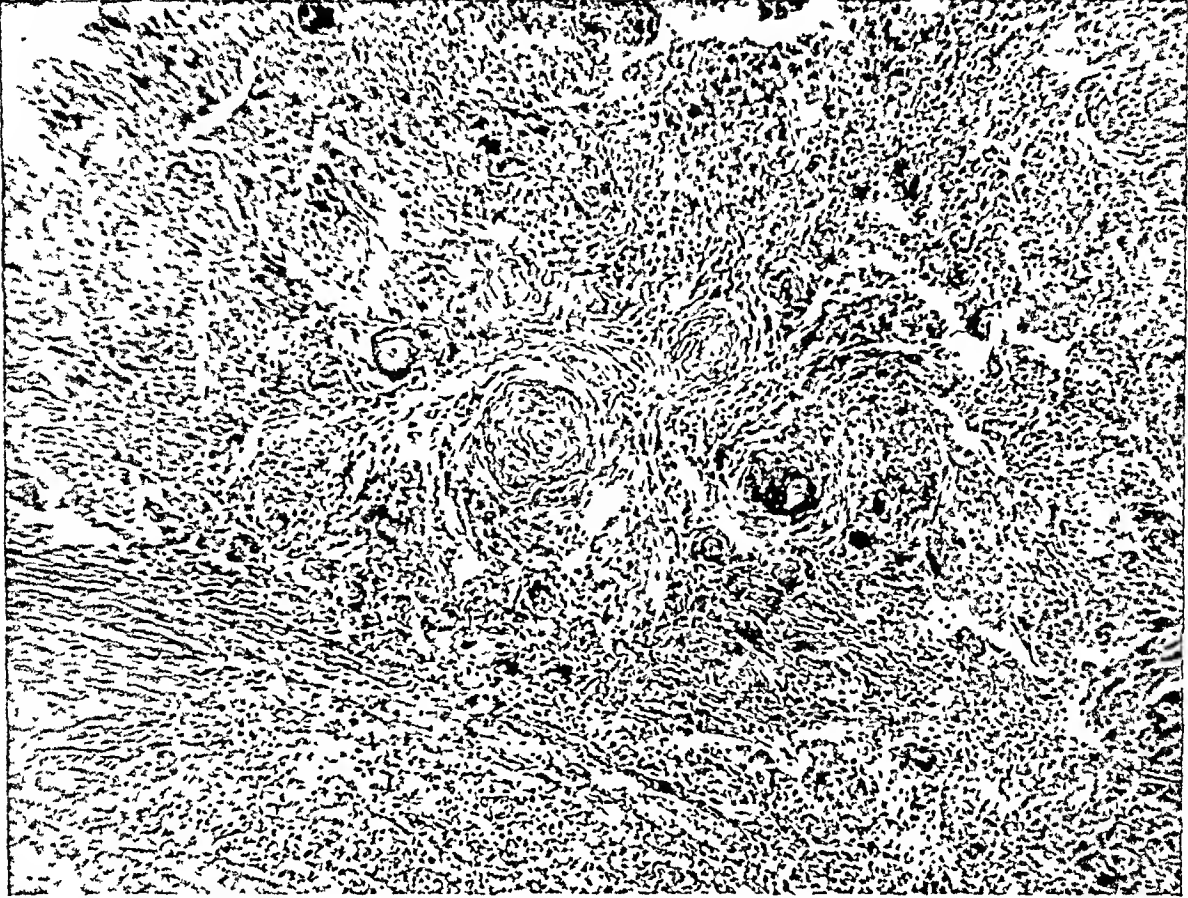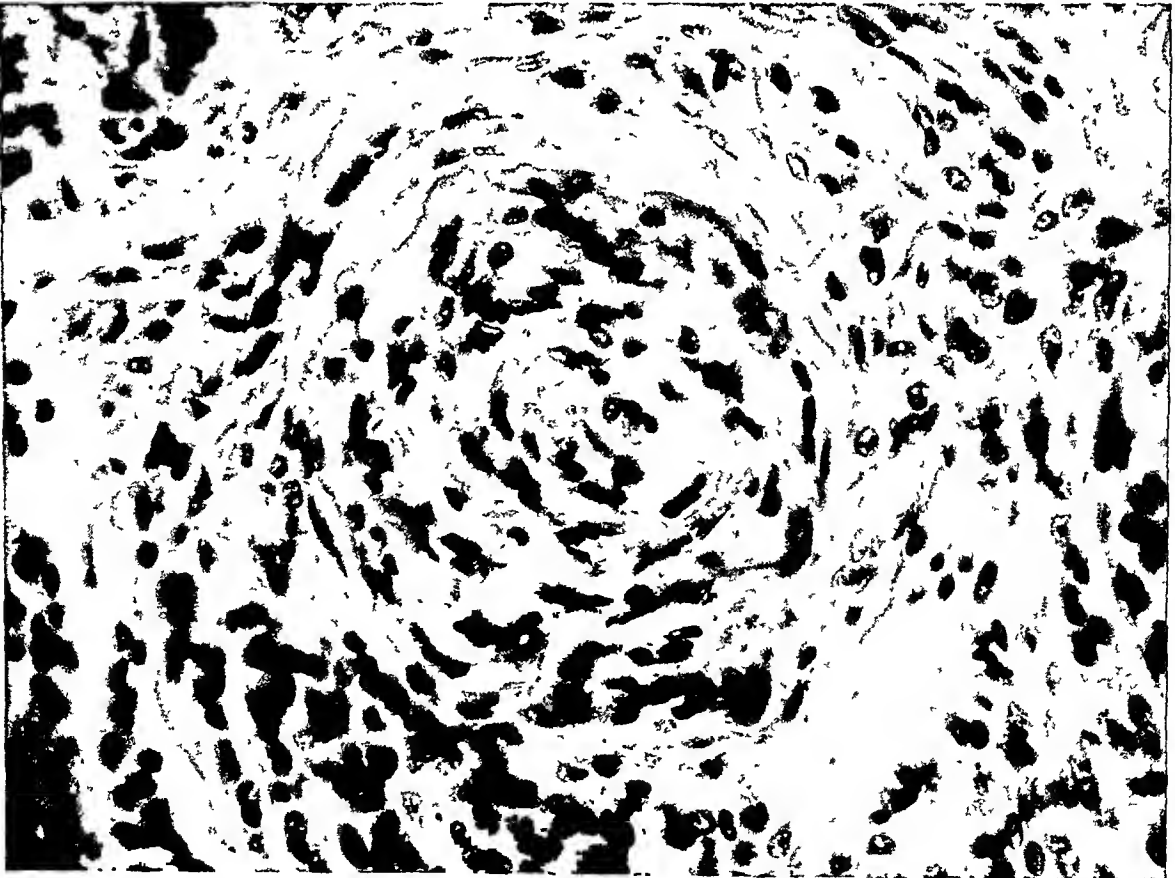
FIG. 5. Bundles of coarse and fine collagenous fibers. Perdrau's silver impregnation. $\times$ 100.

FIG. 6. The center portion of Figure 5. Perdrau's silver impregnation. $\times$ 430.

5
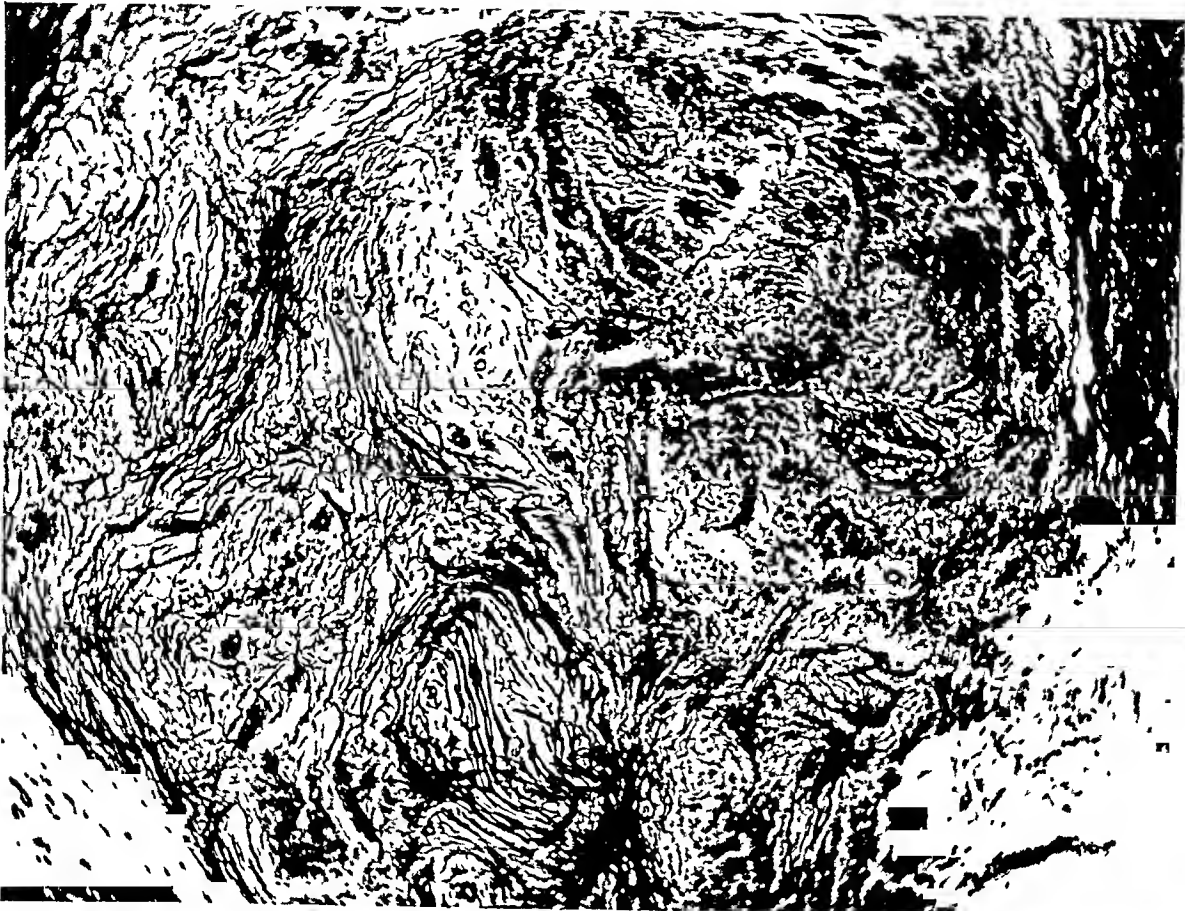
6
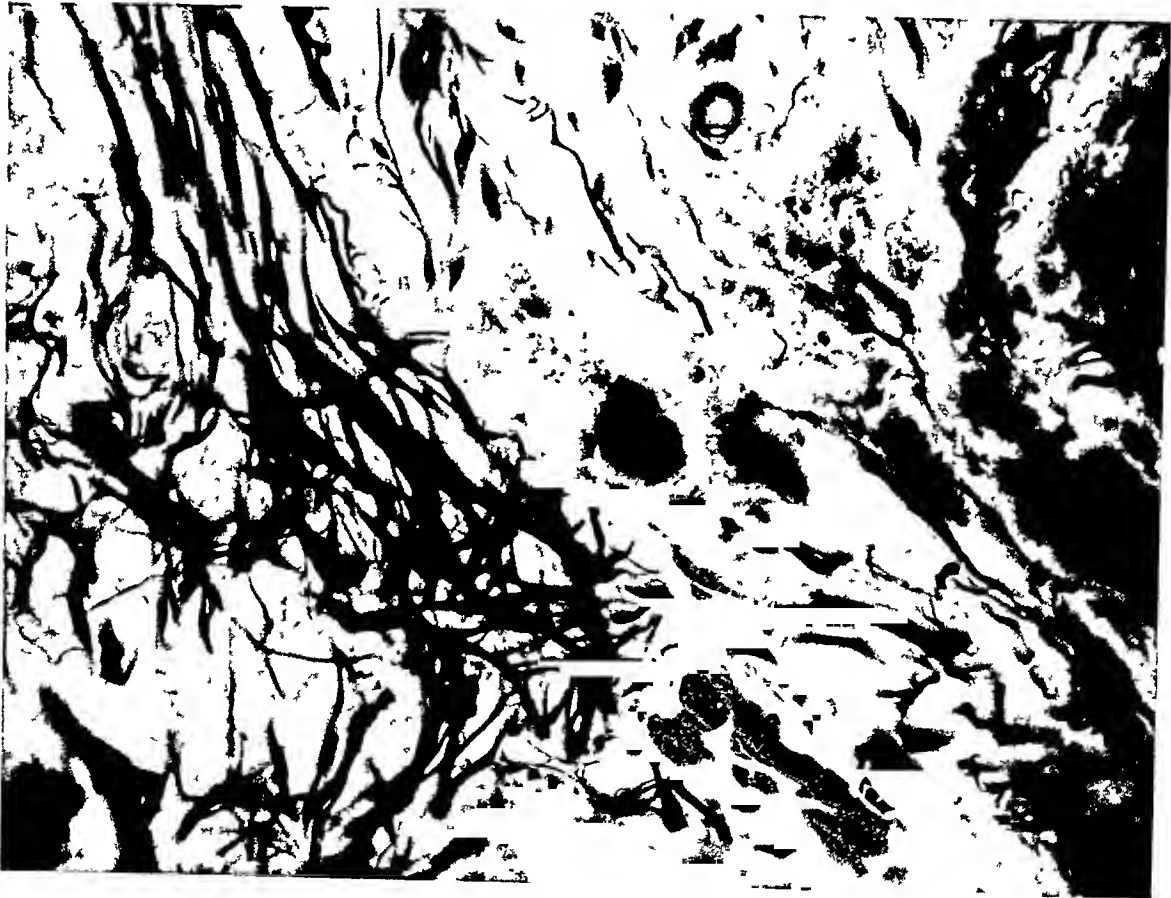
Groszberg and Blumenthal

Subcortical Fibroblastoma of the Brain

OSTEOPETROSIS: ALBERS-SCHÖNBERG DISEASE (MARBLE BONES)

REPORT OF A CASE AND MORPHOLOGIC STUDY *

BERNARD PINES, M.D., and MAX LEDERER, M.D.
(*From the Division of Pathology, Department of Laboratories, Jewish Hospital of Brooklyn, Brooklyn, N.Y.*)

The purpose of this paper is to present a brief clinical history and a detailed pathologic description of a case of marble bones.

In 1904 Albers-Schönberg [1] published a clinico-radiologic study of a case of generalized osteosclerosis. He was able to differentiate this case from a group of apparently similar cases of osteosclerosis associated with primary blood dyscrasias previously reported by other authors. This condition was characterized by increased density of the cortical and medullary portions of the entire osseous system, anemia, and enlargement of the liver, spleen, and lymph nodes. The homogeneous roentgenologic appearance of the bones suggested the name of marble bones (maladie des os marmoreens, Marmorknochenkrankheit, Marmorskelett, morbo marmorea). In 1907 Assmann [2] reported 4 such cases. He conjectured that since this condition appeared to be primarily a disease of the blood-forming organs, osteosclerotic anemia (osteosklerotische Anemie) would be a more appropriate name. Laurell and Wallgren [3] were impressed by the multiple pathologic fractures which occurred in these cases and they introduced the term osteosclerosis fragilis generalisata. In 1926 Karshner [4] collected 18 cases of marble bones from the literature and added 4 of his own. The petrified nature of the bones suggested the term osteopetrosis (stony bone). In 1930 Pirie [5] collected 26 cases from the literature and described 5 new cases. In his cases a drill sank into the bony substance as it would into a mass of chalk. Inasmuch as roentgenologic investigations showed that these bones were of the same density as ordinary chalk, Pirie proposed the name chalky bone for the disease. Among other terms used are lime gout, and congenital osteosclerosis (angeborene Osteosklerose). In 1941 Higinbotham and Alexander [6] collected 131 cases from the literature and added 4 cases. To date 148 cases have been reported.

Many of the cases described cannot be regarded as authentic instances of Albers-Schönberg disease. The clinico-radiologic criteria used to diagnose the condition do not entirely differentiate osteopetrosis from similar, though unrelated, disease entities. A large number of the reported cases are in reality instances of primary disease of the blood with secondary osteosclerosis and myelofibrosis. Zwerg and Laubmann [7] recognized only 55 of the reported cases as instances of

true marble bones. They predicated their conclusions on the following triad of changes: osteosclerosis of the entire skeleton, spontaneous bone fractures, and morphologic alterations in the peripheral blood. However, this triad can be found also in diseases other than marble bones. Further investigation seems necessary for more definite diagnostic criteria.

The only constant diagnostic feature of osteopetrosis is the abnormal increase in the hardness and density of the bones of the skeleton. This, however, is not characteristic of Albers-Schönberg disease alone; it may be seen as a rare form of osteodystrophy associated with other conditions. Heuck [8] was the first to describe a case of generalized osteosclerosis associated with leukemia. Subsequently Neumann,[9] von Jaksch,[10] and others reported similar cases. Jordan and Scott [11] grouped the cases of osseous sclerosis accompanying primary blood dyscrasias according to the alteration of the cells of the peripheral blood. Generalized osteosclerosis has also been found in rare instances of chronic poisoning with fluorine, phosphorus, and strontium. More rarely, it is seen in cases of generalized metastasis of carcinoma to the skeleton.

Secondary anemia is commonly seen in marble bones. Usually it is of a hypochromic, myelophthisic nature, although it may be hyperchromic. The peripheral blood shows immature red blood cells, chiefly normoblasts, and occasional megaloblasts. The platelet count may be normal or low. In children the leukocyte count is high but it may be low in adults. In severe cases of anemia there is a tendency to bleed from the skin and mucous membranes.

Some of the findings are not always constant. The infant or juvenile patient is frail and underdeveloped. The epiphyses appear at the normal time or are slightly delayed and the epiphyseal lines are slow in closing. The growth of the bones in length appears to be normal. The fontanelles remain open longer than usual. Walking, talking, and dentition are delayed. The children appear older than their actual age. The skin is dry, the root of the nose is indented. There are often seen multiple spontaneous bone fractures which produce deformities. Frequently some of the following conditions may be found: Frontal bossing, craniotabes, enlargement of the skull, coxa vara, dorsal scoliosis, beading of the costochondral junctions, deformities of the ribs and chest, enlarged epiphyses of the long bones, bowlegs, syndactylism, achondroplasia, rachitic or pseudorachitic changes, imperfect dentition, overgrowth of the lower jaw, and osteomyelitis of the jaw or of other bones.

Some of the younger patients present symptoms caused by narrow-

ing of the different foramina of the skull. Stenosis of the optic foramina may produce optic atrophy, nystagmus, and blindness. Hydrocephalus and subarachnoid hemorrhages are caused by obstruction of the venous flow at the cranial foramina. Various otologic changes may result from impingement on the auditory nerves.

The signs and symptoms of marble bone disease vary in severity and in time of appearance. They may appear early or late in the course of the disease and may be mild or severe in character. In the infantile and juvenile cases the course is usually more severe and often rapidly fatal. Death may result from anemia, hemorrhage, intercurrent infections, or toxemia secondary to chronic suppurative foci in the bones. In the post-adolescent and adult forms the course is relatively slow and benign. The disease may be carried into adult life without clinical evidence of its presence; it is often detected incidentally after roentgenologic examination of the skeleton. More rarely, attention is called to it by the changes in the configuration of the skull or limbs.

Roentgenologically, the bones of the entire skeleton are homogeneously opaque. The vertebrae, the central part of the pelvis, the base of the skull, the proximal portion of the femur, and the distal ends of the tibia and fibula are more severely affected. The trabecular structure of the bones is partially or completely obliterated and the medullary canal may be reduced in size or absent. The distal ends of the shafts of the long bones and the centers of the flat bones are less sclerotic than the remainder of the skeleton. As a rule, the bones are of normal shape and length but there may be some alteration in their contour. Frequently there is clubbing of the ends of the long bones, especially of the proximal portion of the humerus and the distal portion of the femur. Roentgenologic evidence of rickets may be present in some infants (Karshner,[4] Kudrjawtzewa,[12] Hässler and Krauspe,[13] Kramer and Halpert,[14] McCune and Bradley [15]). Transverse bands of less dense bone running parallel to the epiphyseal plates may be seen in the metaphysis of the long bones and in the short bones. The clinoid processes of the skull are clubbed and thickened. The sella turcica is shallower than normal and the pneumatic structures appear dense.

Systematic chemical analyses of the osseous system in marble bones have been rare. McCune and Bradley [15] were able to find only 5 cases in the literature in which the chemical composition of the bones was analyzed. Metabolic studies of calcium and phosphorus were incomplete. In 1939 Kramer, Yuska, and Steiner [16] reported a case of osteopetrosis in which a comprehensive chemical investigation of the bones was made. Pincus, Gittleman, and Kramer [17] have made similar study of the bones in the case which will be described in this paper. The

conclusions drawn from the study of the above 2 cases are briefly as follows: The calcium and phosphorus content of the bone ash in osteopetrosis is distinctly higher than in normal infants of corresponding age, except in the flat bones of the skull. Calcium and phosphorus are present in the form of tertiary calcium phosphate except near the epiphyses of some of the long bones where a calcium phosphate compound of lower molecular ratio is found. There is diffuse hypermineralization of the bones and a lowered molecular ratio of tertiary calcium phosphate to calcium carbonate, indicating a relative increase in the latter.

Incidence. Osteopetrosis may begin in utero as soon as the process of ossification begins. Pirie [5,18] diagnosed the condition in a case before birth because of the increased density of the bones of the fetus as seen on roentgenologic examination and from the knowledge that other living members of the family suffered from marble bones. The benign form of the disease may begin in early life and may not manifest itself clinically until late. Gortan [19] reported a case of marble bones in a female who lived until 72 years of age. The more typical forms of the disease are seen in infants and juvenile patients. The majority of the cases occur in patients under 10 years of age. Judging from the anamneses, it may be said that some stillbirths and miscarriages may be accounted for by the prenatal fatal form of this disease. There appears to be a slight preponderance in males.

Etiology. There is no satisfactory explanation for the causation of marble bones. Constitution and heredity appear to play a prominent rôle. Many of the cases give a familial history of the disease or a history of parental consanguinity. Sick [20] reported 3 siblings and one cousin who had osteopetrosis; McPeak [21] mentioned 8 cases in 3 generations of one family. In our case, and in other reported instances, the parents were first cousins. Nine of the 40 cases collected by Orel [22] showed parental blood relationship. In a review of 121 cases from the literature van Creveld and Heybroek [23] found that 49 patients gave a familial history of osteopetrosis or of parental consanguinity. This led Kudrjawtzewa [12] and others to conclude that the disease is initiated by a mutation of the germ plasm. The high incidence among relatives suggests the existence of a hereditary factor which is carried as a recessive mendelian character. Some authors suggest that the fatal form of the disease may be carried as a mendelian dominant rather than as a recessive factor.

The cause of Albers-Schönberg disease is still obscure. In the case described by Albers-Schönberg, the patient suffered from lues; only 2 other cases with lues have been reported. Pirie [5,18] believed that Albers-

Schönberg disease is the end-result of an acute, spreading epiphysitis. Laurell and Wallgren,[3] Sick,[20] Lorey and Reye,[24] and others believed that it is due to an imbalanced distribution of calcium. The more accepted view is that it is a result of faulty calcium and phosphorus metabolism. Guassardo[25] suggested that this is due to an abnormal neuro-endocrine innervation. In 1931 Péhu, Policard, and Dufourt[26] found an adenoma of a parathyroid gland in one case of marble bones. Subsequently, generalized osteosclerosis was produced in rats with injections of parathormone. However, no corroborative experimental findings have since been published. Sutro[27] was able to produce osteosclerosis in mice with subcutaneous injections of estrogenic hormone. Pfeiffer, Kirschbaum, and Gardner[28] caused skeletal hyperossification in mice, pigeons, and sparrows with similar injections. The degree of hyperossification depended on the dose and length of administration of the estrogens. Howard and Gonzalez[29] reviewed the literature in reference to the chemico-physiologic aspects of osteopetrosis and found that no apparent intrinsic chemical reaction is responsible for the disease.

Schulze[30] conjectured that osteopetrosis is a healing process of osteomalacia or rickets. This appeared to be substantiated by Kudrjawtzewa[12] who found that the development of Albers-Schönberg disease is preceded by an osteoporotic process. The only other corroborative evidence is the occasional occurrence of rickets in cases of osteopetrosis.

Generalized osteosclerosis has been seen in patients suffering from phosphorus poisoning. These cases also simulate osteopetrosis in some respects, namely, in the necrosis and osteomyelitis of the mandible, disturbance of dentition, and even stratification of the long bones.

More recently, chronic fluorine poisoning has been considered an etiologic factor in marble bones. In 1936 Spéder[31] observed that many animals and human beings living in the phosphatic zones of Northern Africa developed generalized osteosclerosis. This was found to be due to fluorine which was included as calcium fluoride with the phosphorus. He noted similarities in the radiologic and clinical findings in marble bones and fluorine intoxication.

Anatomic studies of marble bones have been rare and incomplete. In 1934 McCune and Bradley[15] found that only 8 completely autopsied cases had been reported. In 1937 Gerstel[32] observed that of 100 cases cited by various authors, only 12 were studied from a pathologic-anatomic viewpoint, and in most of these no particular attention was paid to histopathology.

An analysis of the morphologic studies of marble bones indicates that certain alterations of the skeleton are constant, while others vary

with the degree of development of the disease and the age of the patient. These variations have led to different interpretations of the cellular structure, especially as it relates to the etiology and pathogenesis of the disease. Further histologic study is necessary in order to evaluate the various findings. According to McCune and Bradley,[15] Schmidt,[33] and others, the clinical and morphologic picture seen in infantile and juvenile cases is so different from the adult type as to suggest diseases of different pathogenesis.

REPORT OF CASE

A. E., a white female, was under the observation of the Department of Pediatrics of the Jewish Hospital from birth until death at 18 months. The infant was delivered spontaneously, appeared normal at birth, and weighed 7 lbs., 10 oz. The mother and father were first cousins. Their first child had died at 6 months of unknown causes; one sibling of 7 years was alive and well.

The patient was breast fed and progress was satisfactory until she developed an upper respiratory infection at the age of 4 weeks. At this time a tendency towards nasal bleeding was noted and the liver was palpated two fingersbreadth below the costal margin. The patient recovered uneventfully in 2 weeks.

At 10 weeks of age she was admitted to the hospital because of progressive anorexia, pallor, nasal discharge, and the development of frontal and parietal bosses. Examination revealed a well developed, well nourished, pale infant with a lusty cry. The motion of the extremities was active and unhindered. The temperature was 101°F., the pulse 96, and the respirations 28 per minute. There were prominent changes in the external configuration of the skeleton. Frontal and parietal bosses were seen and craniotabetic areas were felt along the sutures and behind the mastoid regions. The lambdoid sutures were separated 1 cm. The anterior fontanelle measured 3 by 3 cm. and was slightly depressed. The costochondral junctions of the ribs were thickened, angulated, and prominent. The wrists and ankles were widened. The pupils failed to react to light; the right was larger than the left. Funduscopic study revealed a "red retina" with pigmentation and atrophy of the optic nerves. The spleen was palpated 6 cm. below the left costal margin and the liver 4 cm. below the right margin. There was a generalized adenopathy and a number of petechiae were seen on the abdomen. A purulent nasal discharge was present.

The laboratory findings were as follows: Blood chlorides, 378 mg. per 100 cc. of serum; nonprotein nitrogen, 23 mg. per 100 cc. of serum; total protein, 5.2 gm. per 100 cc. of serum; cholesterol, 125 mg. per cent; sugar, 69 mg. per cent; calcium, 8.5 mg.; phosphorus, 2.4 mg.; calcium $\times$ phosphorus, 20; phosphatase, 10.8 Bodansky units; potassium, 19.6 mg.; albumin-globulin ratio, 1.1.

Studies of the peripheral blood showed the following: Hemoglobin, 45 per cent (6.5 gm.); red blood cells, 1,900,000 per cmm.; color index, 1.1; white blood cells, 13,900 per cmm. The differential count revealed 28 per cent polymorphonuclear leukocytes, 2 per cent staff forms, 26 per cent neutrophilic myelocytes, 12 per cent myeloblasts, 2 per cent metamyelocytes, 22 per cent lymphocytes, 8 per cent monocytes. Red blood cell study showed 60 normoblasts per 100 cells; macrocytosis, 4 plus; microcytosis, 2 plus; poikilocytosis, 2 plus; anisocytosis, 2 plus. A bone marrow smear contained very few cells, identified as mitotic red blood cells, myelocytes, myeloblasts, and erythrocytes. It was questioned whether the marrow cavity had been entered.

The urine was negative. Roentgenographic examination of the entire skeleton revealed a general increase in density of all of the bones.

In view of the blood calcium-phosphorus products of 20-32 and the elevated

phosphatase (9-13 Bodansky units), an accompanying rickets was suspected and the patient was treated with repeated small blood transfusions, large doses of viosterol and cevitamic acid. After 3 months of treatment the calcium-phosphorus product rose to 40-50 and the phosphatase decreased. The rickets was considered healed and the blood values subsequently remained at a normal level.

At the age of 4 months the patient had bronchopneumonia. At that time the lower border of the spleen was palpated at the level of the anterior superior iliac spine. The lymph nodes were all enlarged and soft. At 10 months the patient was able to hold up her head but she was unable to sit up without support. Two upper incisor teeth were present and one lower. She was completely blind. At this time she again developed bronchopneumonia from which she recovered in a few weeks. A general muscular atonia was then noted. At 14 months blood and marrow studies continued to show moderate leukogenesis and erythrogenesis, and deficiency in red blood cell maturation. At 16 months the fontanelles were closed, and external rotation and eversion of the lower extremities was noted. The chest appeared to be flattened antero-posteriorly. Severe bleeding from the mouth and nose, which occurred at this time, subsided spontaneously. A similar episode occurred at 18 months and the patient died of uncontrollable hemorrhage.

Roentgenographic examinations of the entire skeleton were repeated at monthly intervals until the time of death. The first, at 3 weeks of age, showed increased density of the bones of the entire skeleton, slight periosteal thickening, and roughening of the metaphyses of the long bones. The skull showed a similar increase in density, especially at the base (Fig. 1). There was wide separation of the bones at the suture lines.

Repeated studies of the skeleton in the ensuing months showed progressively increasing condensation. At 6 months the metaphyses and the epiphyses were developing normally. The long bones were wider than normal and of irregular contour. The cortex of the bones appeared to be less sclerotic than the remainder. Radiolucent striae were seen in the long bones, vertebrae, ribs, and pelvis. Some clubbing of the distal ends of the radius and ulna and of the proximal ends of the tibia was seen (Fig. 2). The ribs also showed increased density; the cortex, like that of the long bones, appeared broad, irregular, and less dense than the medulla. In places the entire width of the rib appeared to have the consistency of the less opaque cortical bone. Just distal to the tubercles of each of the first 10 ribs on both sides, there were single radiolucent striae. The most lateral portions of the bodies of the upper 8 ribs, bilaterally, showed sharp angulation (Fig. 3).

REPORT OF NECROPSY

The body was that of a poorly nourished, white, female infant, 66 cm. long and weighing 9 kg. The skin was pallid; there were numerous red areas around the umbilicus measuring up to 2 cm. in diameter.

The thyroid and parathyroid glands were not unusual.

The trachea and bronchi were filled with frothy fluid. The right lung weighed 70 gm.; the left, 60 gm. Preparations of both lungs showed small areas of extravasated blood in the pleura. The blood vessels were congested. Scattered collections of immature red and white blood cells were seen.

The gastrointestinal tract, liver, and kidneys showed no gross changes. On microscopic examination the sinusoids of the liver and the interstitial capillaries of the kidneys were distended with immature blood cells.

The spleen was firm; it measured 17.5 by 11 by 5 cm. and weighed 460 gm. On the cut surface, the malpighian corpuscles and the fibrous markings were indistinct; the pulp could not be scraped easily. Most of the sinuses were empty; some of them contained immature blood cells.

The lymph nodes were large and firm. The cut surfaces were homogeneous brick red. On microscopic examination the follicles were few and small. The pulp was very cellular. The sinuses were filled with immature blood cells.

On gross examination, the bones were heavier than is normal; they were hard and brittle. The cartilaginous portions were blue-gray and translucent; they cut easily.

The right tibia was slightly club-shaped at both extremities. The epiphyseal line was sharply demarcated and wavy; the zone of provisional calcification was a thin, faintly blue line. The cortical portion of the tibia consisted of dense, homogeneous, gray-white bone which appeared to merge with the compact medullary bone. Beneath the periosteum were a number of red patches.

A number of dorsal and lumbar vertebrae were removed and studied radiographically. The bodies consisted of dense bone. Central and peripheral concentric bands of rarer bone alternated with the more opaque bone. There were also seen oblique radiolucent striae which radiated from the center towards the periphery of the vertebral bodies. The cut surfaces of the vertebrae showed ill defined lines of separation between the cortex and the medulla. In the center of the bodies there was an oval, dark gray area of bone. A similar band of gray bone encircled the vertebra in the region midway between the cortex and the midcentrum. The gray areas in both the tibia and the vertebrae corresponded to radiolucent bands which were noted in the roentgenologic study.

Roentgenograms were taken also of the ribs after removal. The angular deformities previously noted were apparently due to fractures situated about 2.5 cm. lateral to the costochondral junctions. The distal fragments were displaced outward and the angulation measured up to 75°. The lateral portions of the 9th, 10th, and 11th ribs on both sides were also the seat of healed fractures, but there was no angulation. The callus at the sites of all of the fractures consisted of less densified bone of the same radiographic consistency as the cortical bone. On gross examination of the ribs a few small subperiosteal hemorrhages of the pleural superficies were found. The cut surfaces were compact, glistening white, and homogeneous. The sites of angulation consisted of overlapping, wedge-shaped ends of bone incorporated in gray bone callus.

Sections were taken from a number of different areas of each of the following bones: tibia, fibula, dorsal and lumbar vertebrae, and ribs. They were fixed in 10 per cent formalin and in Helly's solution, decalcified in 5 per cent nitric acid, and embedded in both paraffin and celloidin. Hematoxylin and eosin, May-Grünwald's and van Gieson's elastica stains were used.

Histologic Examination of Bones
Tibia

The germinal and proliferating layers of the epiphyseal cartilage of the tibia contained sparsely distributed fusiform and oval chondrocytes. Their cytoplasm was vacuolated and the nuclei were pyknotic. The zone of maturation was narrower than is normal; it consisted of irregularly placed chondrocytes of varying size and shape which lay singly and in isogenous groups in the hyaline matrix. The adjacent cells were aligned in short, irregular columns; at this point they were large, the cytoplasm was foamy, and the nuclei were karyolytic or pyknotic. Between the cells were narrow parallel columns of dense, blue-staining, calcified cartilage matrix. At the irregular medullochondral junction, the epiphyseal cartilage ended sharply. Adjacent to it there was seen in the juxta-epiphyseal portion of the metaphysis an irregular network of short, partly calcified cartilaginous trabeculae. These varied in shape but were of fairly uniform size and arrangement. The trabeculae consisted of an acellular, homogeneous, pale blue, lavender-staining substance, which was often mottled and granular. Occasional swollen chondrocytes appeared within this matrix; they were often encircled and at times completely covered by dense, blue-staining, amorphous calcium.

At the irregular chondro-osseous junction, the primary marrow cavities were small, isolated, and irregular. The marrow consisted of sparse, delicate connective tissue, occasional hematopoietic elements, and rare capillaries. Many of the medullary spaces were empty. The irregular dense blue borders of the cartilaginous trabeculae which lined the marrow cavities represented the fused edges of the original chondrocytic capsules. Their formation is known to follow the invasion of the degenerating cartilage cell columns by the primordial marrow. Upon the surfaces of many of the calcified cartilaginous trabeculae were deposited crescentic layers of dark-pink and pinkish blue-staining osteoid tissue. Many of the marrow cavities were filled with this tissue which showed evidence of having been deposited in concentric rings about capillaries as well as in excentric layers on the surface of the cartilage. The osteoid tissue appeared to form from the delicate fibrocellular marrow. Occasionally the connective tissue cells were seen

within the homogeneous pink substance and gave the appearance of branched osteocytes; in most cases, however, the osteoid tissue was acellular. In some areas, flat osteoblasts lined the surfaces of osteoid islands and produced appositional pre-osseous tissue. More rarely, chrondrocytes within the cartilaginous trabeculae were surrounded by osteoid rings and the cells resembled osteocytes (Fig. 4).

The shaft of the tibia consisted of a central compact network of calcified chondro-osteoid tissue which was enveloped by less dense cortical bone. Nowhere in the shaft was there a definitive bone marrow cavity. The bone was in a quiescent phase and showed no active alteration. Towards the midshaft the medullary bone was dense; the marrow cavities were partially or completely filled with osteoid tissue. The remaining minute myeloid areas contained either delicate connective tissue or were empty.

In the metaphysis of the tibia there was seen an irregular transverse band of bone which differed in appearance from the remainder of the shaft. It consisted of a less dense network of large bony trabeculae and wide marrow spaces. The trabeculae were composed of mixed primitive reticular bone, chondro-osteoid tissue, and early lamellar bone. On their surfaces there was moderately active osteoclastic resorption and osteoblastic deposition of new bone. The myeloid spaces contained both connective tissue and vascular hematopoietic marrow. In places the connective tissue marrow was loose and edematous and in its interstices were seen acidophilic bars of pre-osteal tissue. These portions of the metaphyses corresponded to the transverse bands of diminished density previously noted in the roentgenograms of the skeleton.

At another point in the tibial diaphysis the chondro-osteoid bone was replaced by vascular fibrocellular connective tissue, which was the seat of hemorrhage. Within this tissue were fragments of necrotic bone, many fibroblasts, lymphocytes, plasma cells, histiocytes, brown granular pigment, and a number of foreign body giant cells. Adjacent to this area there was active osteoclastic resorption of the chondro-osteoid tissue with widening of the myeloid spaces. Newly formed primitive bone was undergoing active reconstruction into compact lamellar bone (Fig. 5). This site apparently represented an incomplete fracture which, because of its small size, was not visualized in the roentgenograms of the tibia.

The cortex of the tibia was of varied thickness and presented a variegated appearance. Near the epiphysis it consisted of a single continuous trabecula of reticular bone which lay parallel to the fibrous, avascular periosteum. In the remainder of the shaft it was

composed of parallel and intercommunicating trabeculae which occupied increasingly wider areas from the epiphysis to the midshaft. In the latter situation the cortical bone occupied an area equal to one-third of the width of the entire tibial shaft. It was composed for the greatest part of trabeculae of mixed primitive and lamellar structure. Occasionally the reticular bone predominated and the trabeculae were covered with thin seams of lamellar bone. The large marrow spaces contained varied proportions of delicate connective tissue marrow and moderately vascular cellular marrow. Where the marrow appeared to be more vascular and the seat of hemorrhage, bone alteration appeared to be active. In places, numerous inactive osteoclasts were seen (Fig. 6).

The reticular trabeculae of the cortex were apparently replaced by lamellar bone from the center of the cortex, both inward and outward. This could be deduced from the prominence of lamellar bone in the midportion of this layer, while the outer, subperiosteal trabeculae and the inner endosteal trabeculae appeared to retain their primitive structure in most places.

In isolated areas the cortex consisted of dense, compact, lamellar bone. Numerous lines of growth on the periosteal surface indicated past periodic growth of the bone outwards, while the interrupted trabeculae incorporated in the substance of the compact bone, as well as the partially sclerosed marrow spaces, indicated a previous period of active endosteal bone deposition or inward growth. There was densely cellular fat-free marrow in the medullary spaces.

Vertebrae

Preparations from the bodies of the lumbar and dorsal vertebrae consisted of narrow outer zones of hyaline cartilage which enveloped a dense network of small, irregular, calcified, chondro-osteoid trabeculae. The minute marrow spaces contained delicate fibrous connective tissue. The general structure and appearance of this bone and the process of endochondral ossification resembled that described in the tibia. Within the body of each vertebra were two narrow concentric rings of bone which were analogous in structure to the metaphyseal radiolucent band previously described in the tibia. The outer ring consisted of large interconnected trabeculae of lamellar bone, some of which contained irregular masses of hyaline cartilage. These trabeculae were continuous with the adjacent denser chondro-osteoid tissue or they were separated from the latter by broad medullary spaces (Fig. 7). The marrow spaces contained cellular marrow, large blood vessels, occasional fat cells, and a slight amount of delicate connective tissue.

The inner central ring which was roentgenologically less dense consisted of an uninterrupted band of dense, cortical, lamellar bone which enclosed a large, central, marrow cavity and smaller peripheral spaces containing vascular, cellular marrow. Within the lamellar trabeculae were seen small remnants of reticular bone. A thin seam of primitive bone also lined the peripheries of this lamellar bone ring. Mature bone had replaced the reticular bone which appeared to have occupied this area previously; this process occurred from the center of this zone outward toward the periphery. Large, thick-walled blood vessels ran in straight lines from the perichondrium to the center of the bodies of the vertebrae; they were separated from the chondro-osteoid substance by parallel trabeculae of reticular bone. In their course through the vertebrae the blood vessels appeared to vascularize only the concentric bands of lamellar bone; no branches appeared to enter the chondro-osteoid zones.

Ribs

Preparations from the cartilages, epiphyses, and shafts of the ribs showed morphologic changes similar to those described above. The calcified chondro-osteoid trabeculae which formed the central core of the shaft were of the same architecture but they tended to lie parallel to the surfaces of the ribs. For the most part, the bone was in a quiescent phase. Only in portions of the central chondro-osteoid core where healing, incomplete fractures were seen were there active resorption and reconstruction of bone. The structure in these areas was similar to that previously described at the sites of partial fracture of the tibia. In some portions of the shafts the central chondro-osteoid core was replaced by larger trabeculae of mixed primitive reticular and lamellar bone of the same architecture and distribution as the bone which formed the cortex. This apparently represented complete healing of fractures and it was also found cementing the angulated ends of the fractured ribs. Occasionally, the entire width of the shaft was occupied by large interconnecting trabeculae which were made up of mixed lamellar and chondro-osteoid bone. The irregular distribution of these elements in the trabeculae gave them a mosaic appearance. The large medullary spaces in these areas contained vascular cellular marrow (Fig. 8).

The periosteum of the ribs consisted of a dense hyalinized fibro-elastic outer layer and an inner congested fibrocellular layer; in places it was thickened. Hemorrhagic extravasations were seen between the inner layer of the periosteum and the cortex of the rib. At these sites in the cortex, membranous new bone formation followed on the heels of rapid bone resorption. The structure of the cortex was essentially

similar to that of the tibial cortex. It differed only in that the bone was more sclerotic, compact, and quiescent. In some areas it assumed the pattern of the normal skull; it then consisted of an outer compact table of bone, a central spongy layer, and an inner compact table.

Bone Marrow

It was noted that the myeloid cavities of all of the bones were occupied by different varieties of marrow, depending largely upon the type of bone which surrounded them. For the most part, where the trabeculae were made up of imperfectly composed chondro-osteoid bone the spaces were empty or they contained delicate fibrous connective tissue marrow with a minimal scattering of hematopoietic cells. In the myeloid spaces surrounded by relatively mature lamellar bone the marrow presented a fairly normal appearance. The adipose tissue was easily identified but it was relatively diminished and irregularly distributed. Blood cells in different degrees of maturation, from primordial hematocytes to mature forms, were abundant. Well developed blood vessels, chiefly arterioles with thickened walls, could be seen also in these areas. The spaces, which were bounded on one side by lamellar trabeculae and on the other side by chondro-osteoid tissue, contained a mixture of loosely arranged fibrous tissue cells with oval or vesicular nuclei, a few fat cells, and cellular marrow. Though many erythrocytes and leukocytes were found here, relatively few immature blood cells could be recognized. In some sites, hemorrhage, cell disintegration, and fibrous connective tissue replacement were seen. In some portions of the cortex where reticular bone predominated, mixtures of fibrous and cellular marrow were present; only occasional areas of cellular bone marrow were seen.

DISCUSSION

Histomorphologic study of the skeleton in this case revealed a number of unusual structural changes in the epiphyses, metaphyses, and diaphyses of the long bones and in the bodies of the short bones not heretofore fully described in the literature.

In the preparations of the epiphyses and metaphyses some of the conditions which are necessary for normal growth of bone were not present. The epiphyseal cartilage was inadequately prepared for its part in the mechanism of endochondral ossification and a normal, cellular, vascular, primary marrow was lacking. The different zones of cartilage cell proliferation, maturation, and degeneration were small and poorly developed. The zones of column formation were narrow and irregular; in places they consisted of but two or three cell layers. In

place of the usual vascular marrow at the epiphyses there was seen avascular fibrous connective tissue. This apparently lacked the capillary mesenchymal properties which are necessary for the adequate penetration and dissolution of the short cartilage cell columns, with the result that there was formed at the medullo-chondral junction a scaffolding of short irregular bars of calcified cartilage matrix and minute marrow spaces. Instead of osteoblastic deposition of thin acidophilic osteoid seams on the surfaces of the cartilage spicules, there was seen here an overabundant deposition of calcified osteoid tissue both on the surface of the cartilage bars and in the marrow spaces between them. This tissue appeared to be formed largely by direct fibro-osseous metaplasia of the fibrous connective tissue marrow; less often, it was formed through osteoblastic deposition or through chondro-osseous metaplasia. Instead of the normal, delicate scaffolding of chondro-osteoid spicules and large myeloid spaces containing myelopoietic marrow, there was formed in the metaphyses of this case a dense network of chondro-osteoid tissue and minute marrow spaces containing connective tissue marrow.

Perversion of ossification in the submetaphysis in this case appeared to be due to the absence of osteoclastic and chondroclastic marrow cells. These are essential in resorbing the metaphyseal chondro-osteoid trabeculae in preparation for their replacement by more mature secondary lamellar bone in the submetaphysis. Their absence prevents the alteration of the dense chondro-osteoid tissue so that it remains unchanged in the submetaphysis and no lamellar bone is produced. Mature bone can be formed only when the initial processes of intrachondral bone formation have been completed.

Aberrations from the normal were seen also in the diaphyses of the long bones. Under normal conditions of early bone growth the medullary portion of the shaft consists of mixed trabeculae of chondro-osteoid and lamellar bone and cellular marrow. In the later stages the spongy bone of the submetaphysis appears to merge into the cortex and the diaphyseal spongiosa is resorbed. As a result, the diaphysis of a normal bone ultimately consists of a compact cylindrical cortex and a central definitive marrow cavity. In this case the normal pattern of cancellous bone and of a central marrow cavity was not seen. Instead, it consisted of the hypo-ostotic, sclerotic, calcified chondro-osteoid substance which has been described.

Unusual structural changes were seen also in the periosteal and cortical portions of all bones. According to most of the literature on Albers-Schönberg disease, the cortex is said to be unaffected. In this case the cortex consisted predominantly of layers of parallel trabeculae

of reticular bone and less often of sclerotic lamellar bone. It was irregularly widened in many areas. Occasionally there was seen a concentric, endosteal increase in width without alteration of the outside diameter of the shaft. More often there was also excentric periosteal widening of the cortex with a resultant broadening of portions of the shaft. The cortex of the bones contained less vascular and hematopoietic tissue than is normally found. In places, however, it was vascular and the seat of hemorrhage. Pease, DeSanctis, and Alter[34] reported numerous subperiosteal hemorrhages in their cases. Gerstel[32] described "braune Herde" on the surfaces of almost all bones. These consisted of hemorrhagic areas where the bone was rapidly altering and giant cells were numerous. Similar areas were seen in this case. In still other portions of the cortex numerous osteoclasts were seen on the surfaces of the bone trabeculae but little or no bone resorption was present. There appears to be no anatomic explanation for the inhibited osteoclastic function of the cells in these areas.

When the shaft of a normal bone is once formed, all normal ossification except that at the epiphyseal line takes place in membrane. Membranous ossification is also the most common form of pathologic new bone formation. As a rule, this type of bony growth is not preceded by an intermediate stage of calcification. However, in a number of preparations of the bone in this case there was preliminary calcification of the hyalinized connective tissue of the periosteum and a subsequent alteration and osseous replacement of this calcified fibrous tissue. This type of fibro-osseous metaplasia resembles the process seen in heterotopic ossification which is always preceded by calcification.

The histologic nature of the radiolucent bands has not been commented upon heretofore in the literature. Pirie[5,18] thought that radiologically they resembled the condensed lines of growth that are caused by illness in the young, i.e., the lines that appear following healed rickets, in lead and phosphorus poisoning, in hypothyroidism, and in scurvy. Herscher and Stein[35] conjectured that the alternate lines of varied density may represent alternate progress and recession of the disease. We have noted that these striae stand out in sharp anatomic contrast to the remainder of the bone; they consist of large trabeculae of predominantly mature lamellar bone and wide marrow spaces containing vascular cellular marrow. The relatively normal histologic appearance of these bands favors the concept that they represent periods of remission or attempts at healing. This theory does not, however, explain the fact that the striae are constantly found in the well vascularized regions of the bones which are portals of entry for the nutrient blood vessels. It appears that the bands of radiolucent, relatively

normal bone in osteopetrosis are merely an expression of the relatively normal vascularity of these areas.

It is noteworthy that the skeleton in Albers-Schönberg disease appears generally to be poorly vascularized. The cortex of the bones as well as the regions of healing or healed fractures and the radiolucent striae contain a relatively better blood supply; in these areas the structure of the bone and marrow approaches normal. It appears that diminished vascularity is a factor in the hyperostosis and hypermineralization which are found in marble bones. Leriche and Policard [36,37] and later Jones and Roberts [38] noted that the amount of calcification of tissue depends on the blood supply. It has since been universally accepted that hyperemia leads to decalcification, and bone resorption and anemia to calcification and new bone formation. The lack of normal blood supply in young bone produces no alteration of growth in length. Latarjet [39] ligated the nutrient artery of long bones without affecting their growth. Only in venous stasis, such as occurs in cases of congenital varices as reported by Bier,[40] does elongation of bone occur. Busch [41] showed that when the blood vessels of the fingers are occluded a widening of the long bones occurs; the cortex becomes sclerotic and the marrow cavities are replaced by new bone. The hyperostosis, hypercalcification, and widening of the bones in osteopetrosis may therefore be manifestations of impaired blood circulation.

Bone Fragility in Marble Bones

The osteopsathyrotic propensity in marble bone disease has often been commented upon. Best and Taylor [42] believed that it is due to the disproportion of mineral to organic substance. They stated that the hardness, strength, and rigidity of all bone is conditioned by the balance of organic (fibrous) and inorganic (mineral) constituents, much as the same properties of plaster bandages depend upon the impregnation of cotton mesh with plaster of Paris. The cotton bandage possesses tensile strength but no rigidity, while a plaster cast is rigid but brittle. A proportionate amount of both materials is necessary for strength and resiliency. In osteopetrosis the mineral content of the bones is increased and, as a result, the bones are brittle and easily fractured.

Kramer, Yuska, and Steiner [16] made a chemical analysis of the bones in a case of osteopetrosis to determine whether the nature of the mineral elements as well as their concentration was responsible for bone fragility. The skeleton was found to contain a tertiary calcium phosphate salt similar to that isolated from normal bones; only occasional epiphyseal portions of the diseased bone contained secondary calcium

phosphate salts, a molecular structure which is not found in the normal skeleton. A final conclusion cannot be drawn from the findings in one case, but it appears from their study that the chemical quality of the calcium salt does not play a dominant rôle in the mechanical deficiency of the bones. Further investigation of this problem is indicated.

The relationship of the organic constituents of bone to bone strength has not been dwelt upon in the literature. Examination of the preparations of the skeleton of this case indicated that this factor may play an important rôle. The organic component of normal bone constitutes 30 to 40 per cent of its substance; it consists of the matrix, which is composed of bundles of collagenous fibers arranged in the pattern of lamellae, cementing osseo-albuminous and mucinous material, and osteocytes. The arrangement of the collagenous fibrils into closely cemented plate-like lamellae which run in alternating longitudinal, circular, and oblique directions give this structure its effective maximal strength similar to that attained in the construction of plywood. The skeleton in Albers-Schönberg disease contains relatively little mature lamellar bone; it consists predominantly of chondro-osteoid tissue. The latter does not have the quality, quantity, or arrangement of collagenous fibers and cement substance which is found in normal bone; it is therefore structurally weak. The portions of the cortex which are formed as a result of metaplasia of periosteal connective tissue and of fibrous reticular bone appear to have the same deficiency of the matrix. It is axiomatic that the mechanical value of bone is inversely proportional to the histologic differentiation of the tissue from which it is derived. It may be that hypermineralization and widening of the bones in Albers-Schönberg disease is but a mechanism to compensate for the poor quality of the organic matrix.

In addition to the above factors, the strength and rigidity of bone are also dependent upon the structure and architectural arrangement of the trabeculae which form it. Normal bone trabeculae are shaped in the form of tubes, plates, globes, and cylinders, each of which is fitted for a definite mechanical function. Furthermore, these trabeculae are arranged purposefully in the direction of the lines of maximum pressure or tension acting on a particular bone. The trabeculae of the bones in Albers-Schönberg disease follow no particular shape or pattern and their arrangement does not appear to have been influenced by the trajectories of stress and strain.

Pathogenesis

Controversial opinions have been offered on the pathogenesis of marble bones. Some investigators have contended that it is primarily a

disease of the osseous system and that the bone marrow and blood vessels are affected only secondarily (van Creveld and Heybroek,[23] Lorey and Reye,[24] Bernhardt,[43] Kopylow and Runowa,[44] and others). They have maintained that the mechanical encroachment of the bone upon the marrow produces myelophthisic anemia. To substantiate this opinion they have observed that there is no apparent relationship between the degree of bone alteration and the degree of marrow change and anemia. Many cases with severe osteosclerosis show little or no anemia (Clairmont and Schinz,[45] Howard and Gonzalez [29]) while others with minimal osseous change have marked anemia. Pease, De-Sanctis, and Alter [34] also expressed the belief that osteopetrosis is a disease of osteogenic origin. In support of this idea was the observation that no normal bone was seen in the case reported by them. They reasoned that this differentiated their case from those of osteosclerosis secondary to diseases of the bone marrow, since some normal bone would be found in the latter. Other authors have suggested that marble bones is a disease of the blood-forming organs. Assmann [2] considered it to be some odd form of anemia or leukemia. Klemperer [46] was also of the opinion that it is a dyscrasia of the marrow. He postulated that young bone marrow has potentialities of forming both connective tissue and bone, and, furthermore, that the type of tissue which differentiates from it depends upon the stimulus. He cited the fact that under the influence of x-ray exposure bone marrow shows fibroblastic proclivities and little or no hematopoietic tendency.

It has been conjectured by some authors (Kudrjawtzewa,[12] Hässler and Krauspe,[13] Clifton and Frank,[47], Clifton, Frank, and Freeman,[48] and Grasser [49]) that marble bones is a disease of the undifferentiated mesenchymal anlage of the skeleton and bone marrow. Ontogenetically, the primordial mesenchyme is the common progenitor of both the hematopoietic and osseous systems. From the sclerogenous portion is produced the membranous anlage of the skeletal system which in turn is differentiated into the cartilaginous skeleton. From the myelogenous portion of the mesenchyme is differentiated the vascular bone marrow which assumes an important rôle in the differentiation of the cartilaginous skeleton into the definitive osseous skeleton. The latter process takes place in the first months of development of the bones when the blood vessels of the perichondrium accompanied by undifferentiated myelogenous mesenchyme grow into the hyaline cartilage and forms the primitive marrow cavities.

The histomorphologic changes described in the bones in our case appear to support the theory that marble bones is a disease of the primitive osseomedullary anlage. The clinical and anatomic variations of the

disease are apparently contingent upon the degree of involvement of each of the elements upon whose integrated growth and development depends the structure of the skeleton. It would appear that when the sclerogenous elements are predominantly involved, the disease takes a slow, benign clinical course even though the structure of the bones is greatly altered. When the myelogenous mesenchyme is seriously affected, as is the case in many of the infantile forms of the disease, the course is malignant and rapidly fatal. Between these two extreme forms there exist the varied pictures of marble bones which have been described in the literature.

SUMMARY

Following a discussion of the salient anatomic and roentgenographic features of marble bones and a review of the incidence and etiology of this disease, an illustrative case is presented.

A detailed study of the histomorphology of representative bones of the skeleton revealed changes in the epiphysis, metaphysis, diaphysis, and cortex, which appear to confirm the concept that the pathogenesis of osteopetrosis is related to diseased vascular and osseomedullary anlage.

There is no apparent resemblance of the histologic structure of the bones in osteopetrosis to that in other bone conditions which have been associated in the literature with the pathogenesis of this disease, i.e., rickets, osteomalacia, lues, nonspecific inflammatory disease, phosphorus and fluorine intoxication, and osteosclerosis produced by estrogenic hormones.

Bone fragility in marble bones is due to the disproportion of mineral to organic substance, the poor quality and arrangement of the organic elements of the bones, the uncontrolled variability in size and shape of the bone trabeculae, and their purposeless architectural arrangement.

REFERENCES

1. Albers-Schönberg, H. Röntgenbilder einer seltenen Knockenerkrankung. Ärztlicher Verein in Hamburg. *München. med. Wchnschr.*, 1904, 51, 365. Eine bisher nicht beschriebene Allgemeinerkrankung des Skelettes im Röntgenbild. *Fortschr. a. d. Geb. d. Röntgenstrahlen*, 1907, 11, 261–263.

2. Assmann, H. Beiträge zur osteosklerotischen Anämie. *Beitr. z. path. Anat. u. z. allg. Path.*, 1907, 41, 565–595.

3. Laurell, H., and Wallgren, A. Untersuchungen über einen Fall einer eigenartigen Skeletterkrankung (Osteosclerosis fragilis generalisata). *Upsala läkaref. forh.*, 1920, 25, 309–340.

4. Karshner, R. G. Osteopetrosis. *Am. J. Roentgenol.*, 1926, 16, 405–419.

5. Pirie, A. H. The development of marble bones. *Am. J. Roentgenol.*, 24, 147–153.

6. Higinbotham, N. L., and Alexander, S. F. Osteopetrosis; four cases in one family. *Am. J. Surg.*, 1941, n.s., 53, 444–454.

7. Zwerg, H. G., and Laubmann, W. Die Albers-Schönbergsche Marmorkrankheit. *Ergebn. d. med. Strahlenforsch.*, 1936, 7, 95–136.

8. Heuck, G. Zwei Fälle von Leukämie mit eigenthümlichem Blut- resp. Knockenmarksbefund. *Virchows Arch. f. path. Anat.*, 1879, 78, 475–496.

9. Neumann, E. Ueber leukaemische Knochenaffectionen. *Berl. klin. Wchnschr.*, 1880, 17, 281–284.

10. Von Jaksch, R. Multiple Periostaffection und an myelogene Leukämie mahnender Blutbefund. *Prag. med. Wchnschr.*, 1901, 26, 2–7.

11. Jordan, H. E., and Scott, J. K. A case of osteosclerosis with extensive extramedullary hemopoiesis and a leukemic blood reaction. *Arch. Path.*, 1941, 32, 895–909.

12. Kudrjawtzewa, N. Über Marmorknochenkrankheit. *Arch. f. klin. Chir.*, 1930, 159, 658–687.

13. Hässler, E., and Krauspe. Beobachtungen über generalisierte Knochenerkrankungen des Kindes. *Virchows Arch. f. path. Anat.*, 1933, 290, 193–236.

14. Kramer, B., and Halpert, B. Marble bones. I. Clinicopathologic observations. *Am. J. Dis. Child.*, 1939, 57, 795–808.

15. McCune, D. J., and Bradley, C. Osteopetrosis (marble bones) in an infant. Review of the literature and report of a case. *Am. J. Dis. Child.*, 1934, 48, 949–1000.

16. Kramer, B., Yuska, H., and Steiner, M. M. Marble bones. II. Chemical analysis of bone. *Am. J. Dis. Child.*, 1939, 57, 1044–1057.

17. Pincus, J., Gittleman, I., and Kramer, B. Personal communication.

18. Pirie, A. H. Marble bones. *Am. J. Roentgenol.*, 1933, 30, 618–620.

19. Gortan, M. Un caso di morbo di Albers-Schoenberg. *Boll. e. mem. Soc. piemontese di chir.*, 1932, 2, 769–778.

20. Sick, J. Ueber drei Fälle einer seltenen Skeletterkrankung. (Marmorknochen, Albers-Schönberg). Festschrift zur Feier des 25jährigen Bestehens des Eppendorfer Krankenhauses. L. Voss, Leipzig, 1914.

21. McPeak, C. N. Osteopetrosis; report of eight cases occurring in three generations of one family. *Am. J. Roentgenol.*, 1936, 36, 816–829.

22. Orel, H. Angeborene Missbildungen des Skeletsystems. *Ztschr. f. d. ges. Anat.* (Abt. 2), 1931, 16, 379–386.

23. Van Creveld, S., and Heybroek, N. I. On Albers-Schönberg's disease (marble bones). *Acta paediat.*, 1939–40, 27, 462–494.

24. Lorey and Reye. Über Marmorknochen (Albers-Schönbergsche Krankheit). *Fortschr. a. d. Geb. d. Röntgenstrahlen*, 1923, 30, 35–43.

25. Guassardo, G. Un caso di "morbo marmoreo" (studio clinico, radiologico ed ematologico). *Riv. di clin. pediat.*, 1932, 30, 1531–1558.

26. Péhu, M., Policard, A., and Dufourt, A. L'ostéopétrose ou maladie des os marmoreens. *Presse méd.*, 1931, 39, 999–1003.

27. Sutro, C. J. Effects of subcutaneous injection of estrogen upon skeleton in immature mice. *Proc. Soc. Exper. Biol. & Med.*, 1940, 44, 151–154.

28. Pfeiffer, C. A., Kirschbaum, A., and Gardner, W. U. Relation of estrogen to ossification and the levels of serum calcium and lipoid in the English sparrow, *Passer domesticus*. *Yale J. Biol. & Med.*, 1940–41, 13, 279–284.

29. Howard, C., and Gonzalez, J. Osteopetrosis. Report of a case. *Bull. New York M. Coll., Flower & Fifth Ave. Hosps.*, 1940, 3, 165–171.

30. Schulze, F. Das Wesen des Krankheitsbildes der "Marmorknochen (Albers-Schönberg)." *Arch. f. klin. Chir.*, 1921, 118, 411–438.

31. Spéder, E. L'ostéopétrose de la fluorose phosphatique de l'Afrique du Nord. *Bull. et mém. Soc. de radiol. méd. de France,* 1936, 24, 200–207.

32. Gerstel, G. Über die infantile Form der Marmorknochenkrankheit auf Grund vollständiger Untersuchung des Knochengerüstes. *Frankfurt. Ztschr. f. Path.,* 1937, 51, 23–42.

33. Schmidt, M. B. Ueber osteosklerotische Anämie und Albers-Schönberg'sche Krankheit. *Beitr. z. path. Anat. u. z. allg. Path.,* 1927, 77, 158–173.

34. Pease, M. C., DeSanctis, A. G., and Alter, N. M. Albers-Schönberg's disease. "Marble bones." *Arch. Path.,* 1931, 11, 509–513.

35. Herscher, H., and Stein, J. J. Osteopetrosis associated with Hodgkin's disease. Review of the literature and report of case. *Am. J. Roentgenol.,* 1940, 43, 74-80.

36. Leriche, R., and Policard, A. Physiology of Bone. C. V. Mosby Co., St. Louis, 1928.

37. Leriche, R., and Policard, A. De la chirurgie osseuse. *Presse méd.,* 1926, 34, 242.

38. Jones, R. W., and Roberts, R. E. Calcification, decalcification, and ossification. *Brit. J. Surg.,* 1933–34, 21, 461–499.

39. Latarjet, A. Résultats expérimentaux sur l'accroissement des os en longueur. *Compt. rend. de l'Assoc. d. anat.,* 1912, pp. 72–76.

40. Bier, A. Ueber einige wenig odor gar nicht beachtete Grundfragen der Ernährung. *München. med. Wchnschr.,* 1923, 70, 105–109.

41. Busch, F. Beitrag zur Lehre von der experimentellen Ostitis. *Arch. f. klin. Chir.,* 1879, 21, 331–338.

42. Best, C. H., and Taylor, N. B. The Physiological Basis of Medical Practice. The Williams & Wilkins Co., Baltimore, 1939, ed. 2.

43. Bernhardt, H. Ein Beitrag zur Marmorknochenerkrankung (Albers-Schönberg'sche Krankheit). *Klin. Wchnschr.,* 1926, 5, 415–417.

44. Kopylow, M. B., and Runowa, M. F. Ein Beitrag zur Kenntnis der Marmorknochenkrankheit. *Fortschr. a. d. Geb. d. Röntgenstrahlen,* 1929, 40, 1042–1054.

45. Clairmont, P., and Schinz, H. R. Klinische, röntgenologishe und pathologish-anatomische Beobachtungen zur Marmorknochenerkrankung. *Arch. f. klin. Chir.,* 1924, 132, 347–380.

46. Klemperer, P. Discussion of Pease, DeSanctis, and Alter.[34]

47. Clifton, W. M., and Frank, A. In: Brennemann, J. (ed.) Practice of Pediatrics. W. F. Prior Co., Inc., Hagerstown, Md., 1936, 4, chapter 23, pp. 24–26.

48. Clifton, W. M., Frank A., and Freeman, S. Osteopetrosis (marble bones). *Am. J. Dis. Child.,* 1938, 56, 1020–1036.

49. Grasser, C. H. Ein Fall von Marmorknochenkrankheit mit abweichendem Blutbefund. *Radiolog. Rundschau,* 1938, 7, 174–179.

[*Illustrations follow*]

DESCRIPTION OF PLATES

PLATE 123

FIG. 1. Skull showing condensation of the bones, especially at the base. Suture lines are widely separated.

FIG. 2. Sclerosis of both tibiae and fibulae; somewhat less dense bone in the cortex and in the region of the transverse radiolucent striae. The epiphyses are normal. The ends of the tibiae are clubbed.

FIG. 3. Increased density of the ribs, vertebrae, and pelvis. In the ribs, there are radiolucent striae in the vertebral portions and radiopaque angulation and fractures in the lateral aspects of the bodies. In the ileum, radiolucent striae run parallel to the crest.

1

2

3

Pines and Lederer

Osteopetrosis

PLATE 124

FIG. 4. Section through the epiphyseal cartilage and metaphysis of a tibia. Hematoxylin and eosin stain. × 70.

FIG. 5. Section through the site of a healing fracture of the tibial diaphysis. Of note is the partial replacement of fibrocellular connective tissue by compact primitive and lamellar bone. Hematoxylin and eosin stain. × 65.

FIG. 6. Section through a portion of the cortex of a tibia consisting of parallel interconnecting trabeculae of primitive reticular bone. The marrow spaces are wide. Numerous inactive osteoclasts are seen. Hematoxylin and eosin stain. × 105.
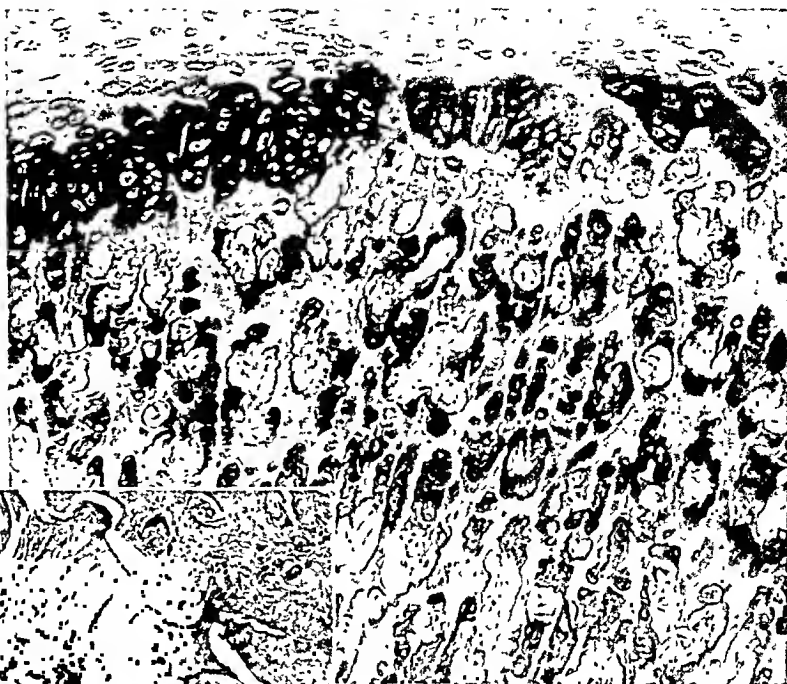
PLATE 125

FIG. 7. Section through the body of a lumbar vertebra showing dense chondro-osteoid tissue on either side of a hypo-ostotic band of bone which consists of large lamellar bone trabeculae and marrow spaces filled with vascular, cellular marrow. The latter area corresponds to the radiolucent striae noted in the roentgenograms of the bones. Hematoxylin and eosin stain. $\times$ 130.

FIG. 8. Section through the body of a rib composed of mixed lamellar and chondro-osteoid elements. The trabeculae have a mosaic appearance. Hematoxylin and eosin stain. $\times$ 120.

Pines and Lederer   Osteopetrosis

III. Pigmentation of the Eyes of Rats Following Ingestion of Silver During Long Periods of Time *

Charles T. Olcott, M.D.

(*From the Department of Pathology, Cornell University Medical College, New York, N.Y.*)

The term argyrosis was introduced by Junge,[1] in 1859, to denote pigmentation of the conjunctiva and other parts of the eye by silver. More recently it has been used in a more general sense to include pigmentation by silver of the skin and various organs and tissues. In the course of an investigation of the effects of silver on the kidney and other organs, several hundred albino rats have been given solutions of silver salts instead of drinking water for varying periods of time that frequently approximated the normal life span of the animals. Their eyes became increasingly dark as this treatment continued. At autopsy, granules, apparently of silver or silver oxide, were deposited especially in the hyaline membrane of the choroid. It is the purpose of this communication (1) to indicate the ocular lesions of argyrosis found in man as reported by others; (2) to describe and correlate the ocular lesions found in the experimental animal during life and at autopsy, with especial reference to pigmentation in the hyaline membrane of the choroid; (3) to indicate points of significant difference between the site of deposition of silver in man and in the experimental animal.

Pigmentation of the human eye by silver usually follows local medication or occupational exposure to salts or organic preparations of silver, but may be part of generalized argyria. In local argyrosis, the conjunctiva and, to a lesser degree, the cornea are especially affected. In 20 human patients on whom otherwise reasonably complete autopsies were performed, the ocular lesions were studied only twice. Frommann[2] demonstrated silver, but his positive findings were limited to the bulbar conjunctiva. Küster[3] added a study of the eye to an autopsy reported by Riemer in 1875 and 1876. This concerned a 43-year-old man who had taken 34 gm. of silver nitrate internally. Küster described silver in finely granular form adjacent to the connective tissue strands and in the vessels of many parts of the eye. It was found in especially large amounts in the tunica propria of the conjunctiva, the subconjunctival tissue, the sclera, the dural sheath of the optic nerve, the capsule of Tenon, and the tendons and interstitial connective tissue of the extrinsic muscles. He could not demonstrate silver in the uveal tract, including the ciliary muscle, because of the

---

deep natural pigmentation of these parts. However, he considered it present, in all probability, at least in the tunica choriocapillaris, the tunica vasculosa, and the interstitial connective tissue of the ciliary body. His reasons for believing that silver was present were in part based on analogy, but even more significant was the fact that he had demonstrated silver in the wall of a ciliary artery and postulated its presence in the entire area of distribution of this vessel. No silver was found in the parts not nourished directly by arteries, namely, the epithelium of the cornea and conjunctiva, the corneal substance, the lens and its capsule, the zonula, and the vitreous humor. Neither was it found in the optic and ciliary nerves, in the retina itself, nor in its vessels.

In a review of many clinical reports in which silver was recognized either by biopsy of the subepithelial tissue of the conjunctiva or by slit lamp or other devices in the cornea, I have found no other reference to pigmentation of the choroid or ciliary body in man by silver.

Study on experimental animals has usually demonstrated only superficial pigmentation by silver. For instance, silver has been applied to the surface of the eye by Gruber,[4] Dieter,[5] and Russo.[6] It has been injected under the conjunctiva by Weymann[7] and Fiore.[8] It has been injected intravenously by Gerlach.[9] By none of these methods was a generalized deposition of silver established. In 1873 Huet[10] reported feeding some rats, one for as long as 14 months, on silver nitrate powder mixed with sugar on bread. Dark pigmentation of the eyes was found during life but silver was not demonstrated on microscopic examination.

EXPERIMENTAL METHODS AND RESULTS

The rats used were albinos of a strain originally coming from Rockland Farms, New City, N.Y., and most of them had been bred in the department for generations. They were given adequate amounts of dog chow, which has been found to be a complete diet for growth and reproduction. Instead of the water given the controls, the experimental animals were given solutions of silver salts. Of the 159 rats whose eyes were studied during life or at autopsy or, generally, in both ways, 143 received a 1:1000 solution of silver nitrate while 16 received a 1:1000 solution of silver chloride held in solution by about 3.5 times as much sodium thiosulfate as silver salt. Eighty-two of the animals were males, 55 were females who had had no litters, and 22 were females who had had at least one litter. The silver solutions were kept in dark bottles and usually given to the rats from about the time of weaning at about 1 month old until their death. In occasional rats, silver was given for a period, after which it was stopped and water sub-

stituted for the rest of the life of the animal. This procedure caused no diminution of pigmentation of the eyes. Following the ingestion of silver salts, there was no apparent change in the duration of life or incidence of infection. The weight of the treated rats was rarely below that of their controls. The incidence of pregnancy and parturition tended to be slightly diminished and lactation was often unsatisfactory, especially in females whose parents had received silver.

Observations During Life

There was rarely any appreciable pigmentation of the skin of the silvered rats. The only significant difference observable during life between those animals receiving silver solutions and those receiving water was the pigmentation developing in the mouth and eyes of the former. The dark color of the eyes was in striking contrast to the bright red appearance of the eyes of untreated albino rats. The incidence of pigmentation was essentially the same in the animals given silver nitrate and in those given silver chloride with sodium thiosulfate. The incidence and degree of pigmentation was similar in males and females whether or not the latter had had litters. An attempt was made to determine objectively the degree of pigmentation of the eyes while the rats were still alive. The pigmentation of the eyes was described as follows: *1 plus,* when the eyes were slightly gray (found 98 times in animals which had received an average of 5.0 gm. of silver salt in an average of 218 days); *2 plus,* when the eyes were more gray than pink (found 84 times in animals which had received an average of 8.9 gm. of silver salt in an average of 373 days); *3 plus,* when the eyes were very dark but had preserved their translucence (found 37 times in rats which had received an average of 10.7 gm. of silver salt in an average of 447 days); *4 plus,* when they were even darker and appeared opaque (8 observations in animals receiving an average of 14.8 gm. of silver salts in an average of 553 days).

Autopsy Findings in Rats

For showing granules of silver in the eye and other organs, Bouin's solution is the most satisfactory fixative, although U.S.P. formaldehyde solution diluted with 9 parts of water can be used. Fixation with Zenker's or other mercuric solution is inapplicable as the necessary removal of the mercury with iodine and sodium thiosulfate [11] removes the silver also. Prepared sections of tissue taken at necropsy from a number of control rats given water and from 139 rats that had been given silver showed the following changes:

Conjunctiva. Silver has never been found in the epithelium of the

conjunctiva. It was regularly found as fine granules along the walls of vessels and in fine strands in the substantia propria of the conjunctiva between the conjunctival epithelium and the extra-ocular muscles at the fornix. As similar strands were stained clearly with Weigert's stain for elastic tissue in a rat receiving water, it is evident that the strands along which the granules of silver were deposited were, in all prob-
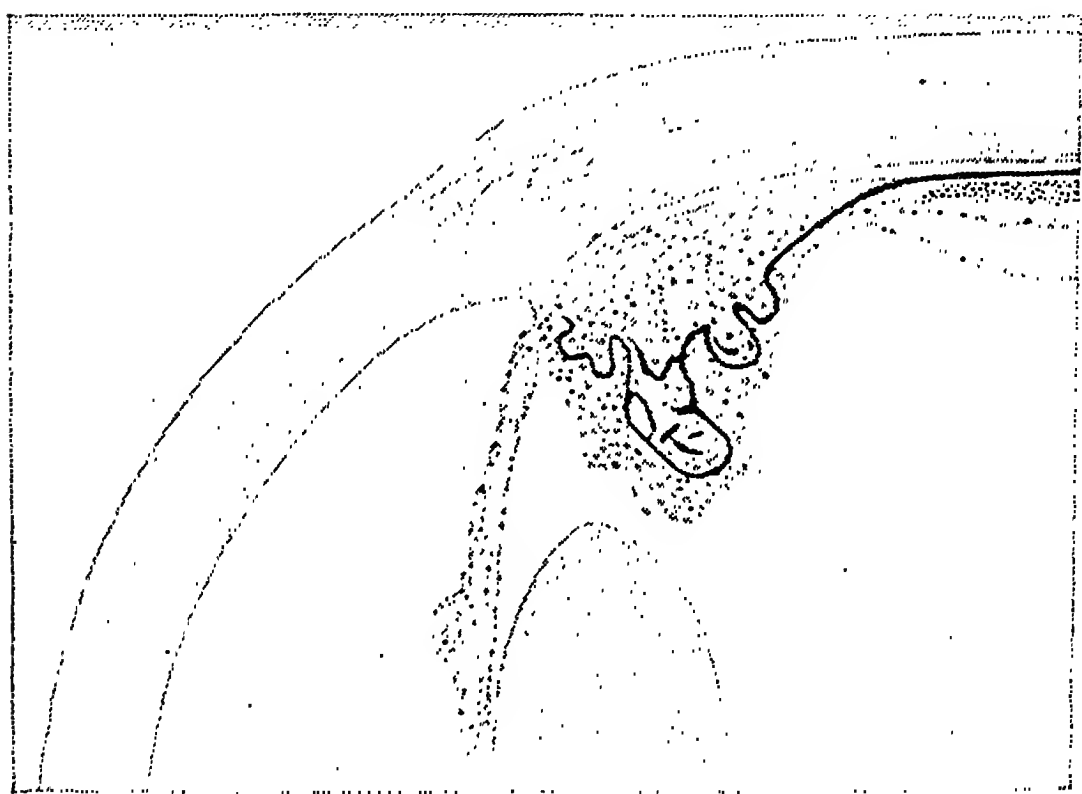
Text-Figure 1. Diagramatic drawing (by Mr. H. Murayama) of a portion of the eye of a rat based on the section shown in Figure 1. The dark line represents the choroid layer and its continuation as the basement membrane of the epithelium of the ciliary body. No silver is demonstrable in the iris or retina.

ability, strands of elastic tissue. No definite strands of elastic tissue were demonstrated in any other part of the eye of the experimental animals. Silver was regularly found to be laid down in small granules around the muscle strands of the extra-ocular muscles. It is not possible to determine whether these granules were deposited along the fine blood vessels or in the endomysium.

Cornea and Sclera. No deposition of silver has been found in the membranes of Bowman or Descemet, or in the corneal endothelium. In a few sections there were a very few fine granules in the substantia propria of the cornea. Also, in a few sections, fine granules of silver were deposited along the vessels and strands of scleral tissue. These

granules, though rare in both tissues, were slightly more abundant in the sclera than in the cornea. From a rat that had received water, an eye stained with Wilder's [12] silver stain showed slightly more pigmentation in Descemet's membrane than was found in the tissues in general. In our experience this technic is very valuable for various purposes but tends to be rather general in its staining so that this pigmentation cannot be considered specific.

Lens. No silver has been recognized either in the capsule or in the substantia propria of the lens.

Choroid. There were usually a few fine granules of silver around the arteries of the vascular layer of the choroid. More numerous and larger granules were found in the capillary layer. It is not clear whether this deposit was essentially pericapillary or whether it was laid down around the fibers on the scleral surface of Bruch's membrane, if such fibers exist in the rat. Certainly, no fibers stained characteristically for elastic tissue.

The site of constant and advanced deposition of silver was the homogeneous layer of the membrane of Bruch. Here the pigment varied from a light lemon color, often containing a few fine, dark granules, to an intense uniform black. In this region an attempt was made to correlate quantitative findings in the ocular sections with those found in other organs, as well as the clinical findings in the eye with the histologic picture. The silver can be removed readily by treating the sections with iodine followed by sodium thiosulfate as used for removing mercury after sublimate fixation.[11] The homogeneous layer of the membrane of Bruch, in an animal that has not received silver, stains like connective tissue with Masson's and Mallory's technic. It does not stain in a way characteristic of elastic tissue with Weigert's stain. When Wilder's [12] silver technic is used, this layer stains darker than any other ocular tissue. Even here, though, the staining is much more fibrillary and less homogeneous than in the rat that has ingested silver for long periods.

Ciliary Body. Pigmentation was regularly present in the membrane underlying the epithelium of the ciliary body. This is of the same magnitude as that found in the homogeneous layer of Bruch's membrane with which it is continuous. No pigment was found in the epithelium itself. The membrane of the control rat stained much less uniformly with Wilder's [12] technic than did those of the rats ingesting silver. No silver was demonstrated clearly in the deeper tissues of the ciliary body.

Iris. No pigmentation was present in the iris.

Retina. No pigmentation due to silver has been found in the layer described in man as the "pigmented layer of the retina." Since all of the rats were albinos, this layer was also devoid of ocular melanin and was entirely unpigmented. No silver has been found in the other layers of the retina.

Optic Nerve. Silver was deposited in moderate amounts in the outer layer of the optic nerve and of its vessels as they entered the eyeball.

Grading of Amount of Deposition of Silver

The amount of silver deposited, with particular reference to the homogeneous layer of the membrane of Bruch, was graded as follows:

One plus, when there were a few, but definite granules.

Two plus, when there was a moderate number of granules. The membrane was usually light yellow between them.

Three plus, when Bruch's membrane was dark brown and the granules formed an almost uninterrupted band.

Four plus, when the membrane was an almost uniform black.

There was good correlation between the pigmentation as observed during life and in histologic sections. Of 111 rats, 59 showed the same degree of pigmentation during life and at autopsy, while in 49 there was a variation of only one grade, with a variation of two grades in the remaining 3 animals. The relative grade of pigment deposition in the eyes of 139 rats was the same as in other organs, chiefly the kidneys, in 25; one grade less in the eyes in 93, and two grades less in the eyes in 21.

It will be seen that the finding in the eyes of rats agree substantially with those described in man by Küster.[3] However, the results in the choroid of the albino rat can be interpreted more precisely than in man because of the absence of any other confusing pigment. In man and the rat the deposition of pigment appears to be determined by the relative amount of vascularity in the various parts of the eye. Certainly, the maximal deposition of silver is present in the especially vascular choroid and ciliary body of the experimental animal. The almost complete lack of deposition of pigment in Descemet's membrane in the rat is in interesting contrast to the usual findings in man.

The localization of silver in the eye is in conformity with the selective localization in vascular areas in other tissues of the body. In material from our rats and from human autopsies, the chief extra-ocular sites where silver is laid down are: the basement membrane of the glomeruli and of the tubules of the kidney, the walls of the portal veins in the liver, the vessels of the thyroid gland, and the choroid membrane of the brain.

SUMMARY

Ocular pigmentation due to the deposition of silver can be recognized during life and at autopsy in rats ingesting silver salts for long periods of time. The amount of pigmentation is directly related to the duration and intensity of the treatment. The zone of greatest pigmentation in the eye is the homogeneous layer of Bruch's membrane of the choroid and its continuation as the basal membrane of the epithelium of the ciliary body.

REFERENCES

1. Junge, E. Argyrose der Conjunctiva. *Arch. f. Ophth.*, 1859, 5, 197–199.
2. Frommann, C. Ein Fall von Argyria mit Silberabscheidungen im Darm, Leber, Nieren und Milz. *Virchows Arch. f. path. Anat.*, 1859, 17, 135–147.
3. Küster, F. Befund an den Augäpfeln und deren Adnexen (addendum to Riemer). *Arch. d. Heilk.*, 1876, 17, 363–366.
4. Gruber, R. On silver-impregnation of the corneal tissue. (Abstract.) *Arch. Ophth.*, 1897, o.s., 26, 426–427.
5. Dieter, W. Ueber Argyrosis conjunctivae und ihre Vermeidung. *Deutsche med. Wchnschr.*, 1929, 55, 1259–1260.
6. Russo, A. Experimental argyrosis of the cornea. (Abstract.) *Am. J. Ophth.*, 1937, s. 3, 20, 1286.
7. Weymann, M. F. Argyrosis of the conjunctiva. (Abstract.) *Am. J. Ophth.*, 1933, s. 3, 16, 850.
8. Fiore, T. Researches on the chemistry of argyrosis. (Abstract.) *Am. J. Ophth.*, 1936, s. 3, 19, 85.
9. Gerlach, W. Zur Frage der Argyrosen, insbesondere des Auges. *Klin. Wchnschr.*, 1934, 13, 797.
10. Huet. Recherches sur l'argyrie. *J. de l'anat. et physiol.*, 1873, 9, 408–434.
11. Mallory, F. B. Pathological Technique. W. B. Saunders, Philadelphia & London, 1938, p. 41.
12. Wilder, H. C. An improved technique for silver impregnation of reticulum fibers. *Am. J. Path.*, 1935, 11, 817–819.
13. Olcott, C. T., and Riker, W. F., Jr. Experimental argyrosis. II. Treatment of rats receiving silver with 2,3-dimercaptopropanol (BAL). *Science*, 1947, 105, 67.

[Illustrations follow]

DESCRIPTION OF PLATE

PLATE 126

FIG. 1. Photomicrograph from an eye of a male rat, 16 months old, that received a total of 14.3 gm. of silver chloride in sodium thiosulfate in 444 days. Pigmentation of the choroid and basement membrane of the ciliary body was graded 3 plus.

FIG. 2. Photomicrograph from an eye of a female rat, 20 months old, that received a total of 12.2 gm. of silver chloride in sodium thiosulfate in 514 days. Injection of this rat with 2,3-dimercaptopropanol (BAL) caused no apparent diminution of pigmentation during life or at autopsy.[13] The clear layer between the nuclei of the retina and the choroid corresponds to the pigmented layer of the retina of man.

PLATE 126
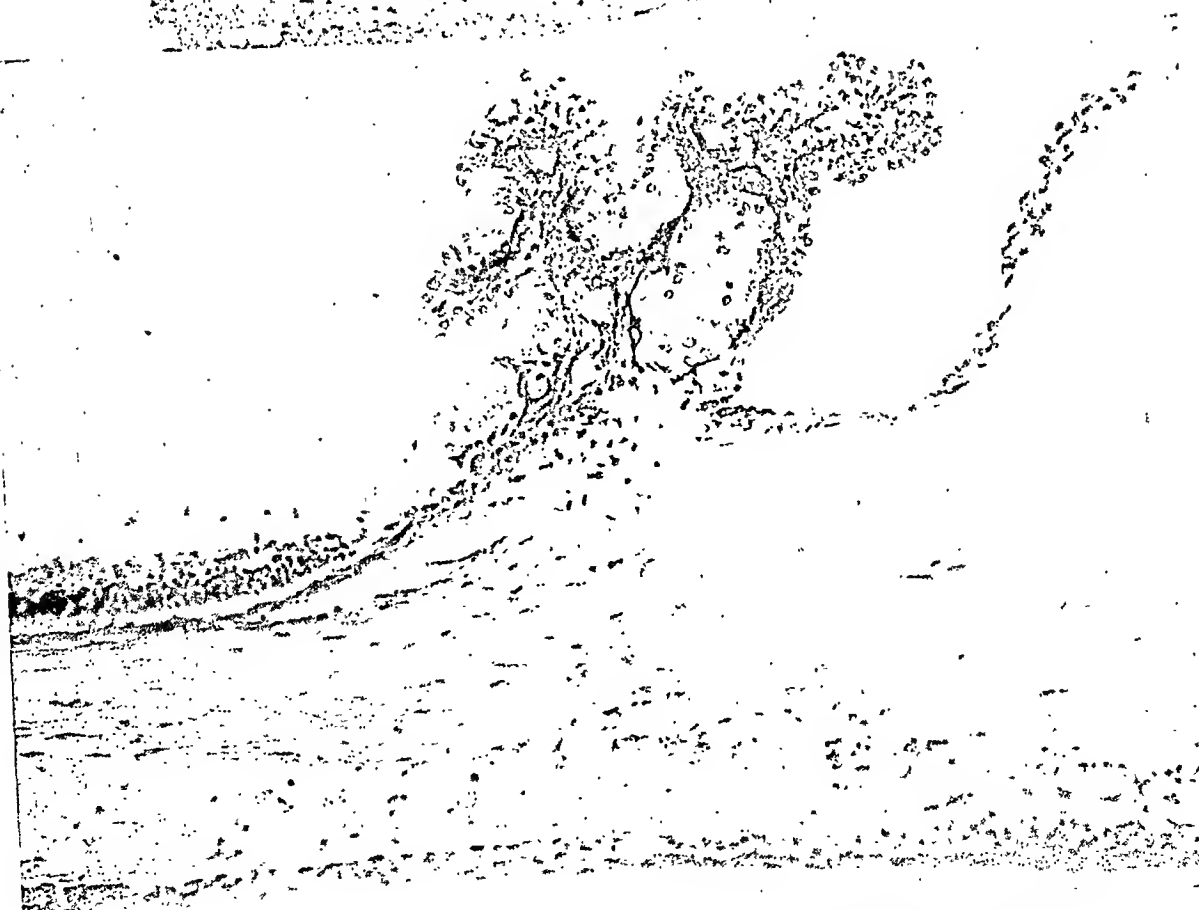
Experimental Argyrosis of the Eyes

Olcott

THE RESORPTION OF ARTERIAL ATHEROMATOUS DEPOSITS IN WASTING DISEASE *

SIGMUND L. WILENS, M.D.

(From the Departments of Pathology, New York University College of Medicine and Bellevue Hospital, New York 16, N.Y.)

A statistical analysis [1] has revealed that at necropsy severe atherosclerosis is at least twice as common in obese as in undernourished persons 35 years of age or older. This relationship proved to be independent of sex, hypertension, and diabetes. A large number of those found to be undernourished at necropsy had been well nourished or even obese prior to the onset of terminal wasting disease. If the degree of atherosclerosis that had developed in those cases was unchanged during the final illness, the relationship between atherosclerosis and overnutrition must be even more striking than was indicated; for if these individuals had developed marked atheromatous lesions during periods of average or overnutrition, their inclusion among the undernourished in a necropsy series would raise the incidence above its real value.

The alternative explanation is that during a period of marked weight loss, significant resorption of previously formed atheromatous deposits may occur. There is little evidence that such lesions in man are reversible. It is not unreasonable to suppose that early deposits consisting largely of lipids might be resorbed under suitable circumstances. Hyalinized or calcified intimal plaques may harbor resorbable material in their depths. Withdrawal of such deposits could lead to diminution in size of even advanced lesions.

The persistence of intimal deposits of cholesterol in the arteries of rabbits depends upon the continued feeding of cholesterol-rich diets (Krylov,[2] Anitschkow [3]). These deposits in rabbits closely simulate early human lesions. This suggests that the early human lesion is not necessarily permanent. Krisch,[4] Zinserling,[5] Aschoff,[6] and Ophüls [7] believed that the intimal lipid streaks noted frequently at necropsy in the aortas of infants and children may undergo spontaneous regression. However, because of the proximal location and diffuse character of such deposits, their relationship to adult atheromatosis has been disputed.[8] Leary [9] stated that cholesterol deposits in the atheromatous lesions of man may undergo lysis. The literature on this subject has been discussed in detail in the review by Hueper.[10]

The importance of determining whether or not atherosclerotic lesions are reversible at all is self-evident. If spontaneous resorption can

occur, it is·possible that the conditions leading to this event might be simulated. The present study was undertaken to determine if appreciable resorption of atheromatous lesions in the aorta and coronary arteries occurs in patients who lose considerable weight during a terminal illness.

MATERIALS AND METHODS

Kodachrome photographic slides were prepared of the intimal surfaces of the aortas and main branches of the coronary arteries obtained at necropsy from 104 patients in Bellevue Hospital. Extensively calcified or narrowed portions of the coronary vessels were photographed in cross section. With a few exceptions photographs of both the aorta and the coronary arteries were available from each patient. In all, the state of nutrition during health, the duration of the final illness, and the amount of weight lost during that period were known.

The analysis was restricted to those from 40 to 60 years of age at death because it is usually at about 40 years of age that·a significant degree of atherosclerosis becomes manifest, while at 60 years a high incidence is observed. Older patients were excluded because in them there might have been a long interval between the acquisition of some of the atherosclerotic plaques and death. Moreover, the state of nutrition of the individual may have been greatly altered in the interim.

The data concerning weight loss were supplied largely by the patients. Evidence of recent weight loss as noted on physical examination was used as a check. It is likely that some patients were unaware of, or denied, minor degrees of recent weight loss. Consequently, in the patients listed as not having lost weight, there may be some error. Striking quantitative differences in the amount of atherosclerosis observed in those listed as not having lost weight and those who had lost weight are, however, of significance for comparative purposes.

Thirty-nine of the group in which the coronary arteries were studied and 41 of the group in which the aortas were studied had lost from 15 to 110 lbs. in a period of from 1 to 12 months preceding death. The remainder in each group had lost less than 10 lbs. prior to death. Two in the coronary artery group and 3 in the aorta group had gained a small amount of weight terminally.

The evaluation of the state of nutrition at necropsy was based on the amount of adipose tissue deposited in the subcutaneous, mesenteric, omental, retroperitoneal, and perirenal regions. The standards were the same as those used in a previous analysis.[1]

The degree of atherosclerosis was measured by arranging the kodachrome slides, as unknowns, in sequence, using the extent of intimal surface covered by plaques of various types as the chief criterion.

Where the process was of approximately equal extent in any two, the one showing more advanced lesions, namely, those that were hyalinized, calcified or ulcerated, was considered to have the greater degree of involvement. The series was then divided into four groups of 26 cases each. The first group, with the least change, will be referred to as showing "minimal" lesions; the second group will be listed as "mild"; the third, as "moderate"; the fourth, as "severe." The sorting-out process was repeated three times and, although there were minor shifts in position within each group, in only isolated instances were these great enough to displace any individual case from one major group to another.

RESULTS

The Relation of Terminal Weight Loss and Nutrition to Atherosclerosis

In Table I the number of cases of minimal, mild, moderate, and severe atherosclerosis of the coronary arteries found in the obese, average, and poorly nourished groups and the association with weight loss in each category are indicated. The relationship between nutrition and severity of atherosclerosis is striking. Twelve of the 24 obese, but

TABLE I

The Relation of Terminal Weight Loss and Nutrition to Coronary Atherosclerosis

| Nutrition | Weight loss | Degree of atherosclerosis | | | | Total |
		Group I Minimal	Group II Mild	Group III Moderate	Group IV Severe	
Obese	With	0	0	0	3	3
	Without	2	3	7	9	21
Average	With	3	3	1	3	10
	Without	4	7	11	9	31
Poor	With	9	8	7	2	26
	Without	8	5	0	0	13
Totals	With	12	11	8	8	39
	Without	14	15	18	18	65

only 2 of the 39 poorly nourished persons had severe atherosclerosis of the coronary arteries. This relationship is even more pronounced than was reported in an earlier analysis [1] on a larger series of less well controlled cases. As in the earlier report, if the hypertensive cases are excluded, a relationship between severe atherosclerosis and overnutrition is still demonstrable. Both of the poorly nourished persons but only 6 of the 12 obese individuals with severe atherosclerosis had high blood pressures. Similarly, in this small series as in the larger one previously reported, the relationship between atherosclerosis and nutrition is independent of sex and age.

Among the 13 poorly nourished persons who had not lost weight terminally and who therefore were presumably poorly nourished for long periods of time, there were no examples of either severe or moderate atherosclerosis. All 9 of the poorly nourished persons who had moderate or severe atherosclerosis of the coronary arteries had lost weight prior to death and probably had been previously well nourished. It is thus seen that terminal weight loss tends to obscure the nutritional factor in the pathogenesis of atherosclerosis.

There were 5 obese persons in the series who had not lost weight, but who, nevertheless, had only mild or minimal atherosclerotic lesions. Therefore, obesity is not invariably associated with severe atherosclerosis. Furthermore, 2 of these 5 were not only obese but had hypertension as well. However, they were relatively young; one was a 45-year-old male and the other a 46-year-old female. The remaining 3 obese, nonhypertensive persons with little atherosclerosis of the coronary arteries were a 51-year-old male and 2 females, each 59 years old.

It may be noted also that 17 poorly nourished persons with weight loss prior to death had only minimal or mild atherosclerosis of the coronary arteries. When the degree of atherosclerosis was compared in all persons with or without terminal weight loss, a relatively large proportion of those with weight loss showed little atherosclerotic change. When the poorly nourished persons without terminal weight loss were excluded from this comparison on the grounds that they probably never had had significant atherosclerotic lesions, this relationship was strongly accentuated. Thus only 16 of 52 well nourished or obese persons (31 per cent) without terminal weight loss had minimal or mild atherosclerosis whereas 23 of 39 (59 per cent) with weight loss fell into similar categories.

The results of a similar analysis made on the aorta (Table II) are

TABLE II

The Relation of Terminal Weight Loss and Nutrition to Aortic Atherosclerosis

| Nutrition | Weight loss | Degree of atherosclerosis | | | | Total |
		Group I Minimal	Group II Mild	Group III Moderate	Group IV Severe	
Obese	With	0	0	1	2	3
	Without	1	4	8	8	21
Average	With	4	3	2	0	9
	Without	4	8	8	10	30
Poor	With	10	8	5	6	29
	Without	7	3	2	0	12
Totals	With	14	11	8	8	41
	Without	12	15	18	18	63

almost identical, indicating that the relationship between atherosclerosis, state of nutrition, and terminal weight loss is not peculiar to any one artery. Of 29 poorly nourished persons who had lost considerable weight before death, 18 (62 per cent) had only minimal or mild atherosclerotic lesions in the aorta. Of 51 persons who were obese or of average weight and who had not lost weight prior to death, 17 (33 per cent) had only minimal or mild lesions.

There is thus suggestive evidence that less severe degrees of atherosclerosis in both the aorta and coronary arteries are observed in persons who had lost weight prior to death than in those who had not. The implication of this finding is that there may be significant regression of at least certain types of lesions in association with wasting disease.

The Relation of Macroscopically Visible Intimal Lipid Deposits to State of Nutrition and Wasting Disease

As previously noted, it is reasonable to expect that the lipid component of intimal lesions might undergo resorption more readily than hyaline or calcific material. For this reason, the kodachrome slides were rearranged in graded sequence according to the amount of visible lipid in the form of diffuse yellowish intimal deposits, as streaks, flecks, or circumscribed mounds. Each series was again subdivided into four equal groups of 26, designated consecutively as "minimal," "mild," "moderate," and "severe." In the rearrangement the presence of hyalinized, calcified, ulcerated, or hemorrhagic areas was disregarded. The new groups differed considerably from the original based on grading all types of atherosclerotic lesions.

In Table III the relation of the amount of grossly visible lipid in the

TABLE III

The Relation of Terminal Weight Loss and Nutrition to Lipid Deposits in Coronary Arteries

Nutrition	Weight loss	Degree of lipid deposition				Total
		Group I Minimal	Group II Mild	Group III Moderate	Group IV Severe	
Obese	With	0	0	2	1	3
	Without	1	3	6	11	21
Average	With	3	4	0	3	10
	Without	7	4	12	8	31
Poor	With	9	10	4	3	26
	Without	6	5	2	0	13
Totals	With	12	14	6	7	39
	Without	14	12	20	19	65

coronary arteries to state of nutrition and terminal weight loss is shown. Large amounts were found more often in the coronary arteries of persons who were obese or of average weight than in those who were poorly nourished. There were 20 obese persons and 23 of average nutritional state who had moderate or marked intimal lipid deposits, and in each of these groups only 3 had lost weight terminally. In the poorly nourished group only 2 without terminal weight loss showed moderate intimal lipid deposits and none had marked deposits.

Of the 26 poorly nourished persons who had lost weight terminally because of wasting disease, 19 had minimal or mild and only 7 more extensive deposits of intimal lipid. It may be inferred, therefore, that grossly appreciable depletion of lipid deposits usually occurs during a period of weight loss lasting several months.

TABLE IV

The Relation of Terminal Weight Loss and Nutrition to Lipid Deposits in Aortas

| Nutrition | Weight loss | Degree of lipid deposition | | | | Total |
		Group I Minimal	Group II Mild	Group III Moderate	Group IV Severe	
Obese	With	0	1	0	2	3
	Without	2	3	7	9	21
Average	With	3	3	2	1	9
	Without	2	7	10	11	30
Poor	With	9	11	6	3	29
	Without	10	1	1	0	12
Totals	With	12	15	8	6	41
	Without	14	11	18	20	63

There were, however, 3 obese persons and 3 of average nutritional state as well as the 7 poorly nourished persons who, despite terminal weight loss, continued to show moderate or marked lipid deposits. The data available do not indicate that this group of 13 that failed to show reduction of intimal lipid had lost less weight or that the average period of weight loss was shorter or longer than in the group of 26 with terminal weight loss that apparently did have significant withdrawal of lipid deposits. Moreover, hypertension does not appear to be a factor in the failure of resorption of lipid. Only 4 of the 13 with moderate or marked amounts of persistent lipid had definitely elevated blood pressures.

Lipid deposits in the intima of the aorta bear approximately the same relation to state of nutrition and terminal weight loss as those observed in the coronary arteries. Relatively few obese or well nourished persons had only scanty lipid deposits in the aorta; relatively few poorly nourished ones had large amounts (Table IV). Of 41 persons, most of whom presumably had been well nourished or obese prior to

a terminal wasting disease, 27 had only minimal or mild deposits of lipid in the aortic intima. The remainder still had large intimal deposits.

It may be concluded, therefore, that during periods of weight loss, lipid deposits in the intima of arteries usually, but not invariably, tend to undergo resorption.

There are some differences in the distribution and character of lipid deposits in those who have lost weight terminally as opposed to those who have not. As a rule, the lipid deposits in well nourished or obese persons who have not lost weight tend to be sharply outlined or circumscribed, to project above the surrounding intimal surface, to be superficially located close to the endothelial surface, and to be fairly thick and opaque. Often in this group, the margins of older hyalinized plaques are surrounded by a narrow rim of yellow lipid.

On the other hand, in persons with wasting disease, lipid deposits are often thin and partly translucent as well as being scanty in amount. They tend to lie deep in the intima and to have frayed or indefinite borders. The intimal surface often is not raised in the areas of lipid deposit. Lipid deposits usually are not seen at the periphery of hyalinized plaques. However, these features are by no means constant.

The Relation of Hyaline and Calcific Intimal Plaques to Nutritional State and Terminal Weight Loss

There is little reason to believe that either hyaline or calcified plaques can undergo complete resolution or even partial regression except over long periods of time. Nevertheless, the series was analyzed to see if any appreciable difference in the number and size of such lesions could be observed in the arteries of those who had lost weight terminally as contrasted with those who had not. The kodachrome slides again were arranged in sequence according to the extent of hyaline and calcific plaque formation so that four new groups of 26 cases for each series were obtained.

It is apparent (Table V) that terminal weight loss had little effect on the extent of intimal involvement of the coronary arteries by hyalinized or calcified plaques. The cases with terminal weight loss were distributed almost evenly in the four groups. While there were 20 persons with terminal weight loss who had only minimal or mild formation of hyaline or calcific plaques, there were also 19 who had moderate or marked changes of this type. Analysis of the aortic series in the same manner revealed a similar negative correlation between weight loss and hyaline and calcific plaques. The data of this analysis are therefore not presented.

The results in Table V indicate, nevertheless, that a relation between

the general state of nutrition and the formation of these lesions does exist. Thus 13 of 21 obese persons without terminal weight loss had moderate or marked·hyaline and calcific plaques. Only one of the 13 poorly nourished persons without terminal weight loss showed moderate hyaline or calcific plaques in the coronary arteries, and none had severe lesions. However, when terminal weight loss is ignored and the state of nutrition as observed at necropsy alone is considered, this relationship is obscured. Twelve of 39 poorly nourished persons, including both those with protracted undernutrition and those with terminal

TABLE V

The Relation of Terminal Weight Loss and Nutrition to Hyaline and Calcific Plaques in Coronary Arteries

Nutrition	Weight loss	Degree of hyaline and calcific plaque formation				Total
		Group I Minimal	Group II Mild	Group III Moderate	Group IV Severe	
Obese	With	0	0	1	2	3
	Without	1	7	5	8	21
Average	With	4	1	2	3	10
	Without	7	5	9	10	31
Poor	With	8	7	8	3	26
	Without	6	6	1	0	13
Totals	With	12	8	11	8	39
	Without	14	18	15	18	65

weight loss, had moderate or severe formation of hyaline and calcific plaques.

The finding that both lipid deposits and hyalinized and calcified plaques bear the same relationship to the general state of nutrition is further evidence that these lesions, as commonly believed, are related to one another, and that hyalinization and calcification occur in areas that were originally the site of simple lipid deposits.

In Text-Figure I the incidence of moderate and severe atheromatous lesions of various types in those who had lost weight terminally and in those who had not is contrasted graphically.

The Relation of Terminal Weight Loss to the Histologic Appearance of Atheromatous Lesions

Histologic sections of Zenker's-fixed, paraffin-embedded tissues stained with hematoxylin and eosin were studied from most of the aortas and many of the coronary arteries used in this analysis. Since in any one artery the lesions are likely to vary considerably in character and distribution, random sections provide an inadequate sample of

the atherosclerotic process as a whole. It is, therefore, not possible to present quantitatively the histologic differences observed. In general, however, the gross observations were confirmed, namely, that the atheromatous lesions were less pronounced in those who had lost weight terminally than in those who had not. This was true of almost all features of the process but more particularly in respect to lipid deposition.

Lipids can be identified in atheromatous lesions chiefly in three
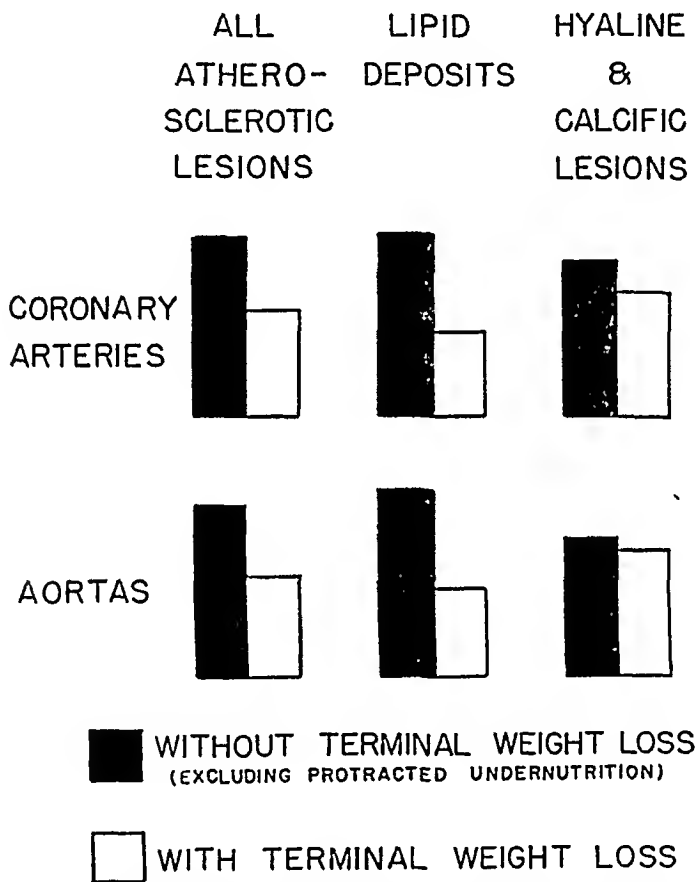
Text-Figure 1. The incidence, in percentage, of moderate and severe atheromatous lesions of various types in the aortas and coronary arteries of persons who had not lost weight prior to death as compared with those who had lost weight terminally.

forms: contained within phagocytes, as cholesterol crystals, and impregnating diffusely the amorphous material in the depths of large intimal plaques. As a rule, less lipid in each of these three forms was noted in the arteries of those who had lost weight terminally than in the arteries of those who had not. The most consistent difference, however, was in the number, size, and distribution of lipid-containing phagocytes. In the group with wasting disease these were generally few in number, relatively small, and often scattered as single cells in crev-

ices of dense or hyalinized connective tissue. In the group without terminal weight loss, these cells were often numerous, grouped in clusters, swollen with numerous fine droplets of fat, and superficially located just beneath the endothelial surface or at the margins of large intimal plaques. These differences were by no means constant or uniform. Cholesterol crystal spaces were found less often in the amorphous material contained at the base of large intimal plaques in those with terminal weight loss than in those without terminal weight loss. The amount of amorphous material deposited in the depths of intimal plaques was also generally less. On the other hand, diffuse intimal thickening without discrete plaque formation was often more conspicuous in those who had lost weight terminally. No conspicuous difference in the amount of hyalinized intimal connective tissue was noted, although other regressive changes such as calcification, "ulceration," chronic inflammation, increased vascularity, and hemorrhage were less often observed in the group with terminal weight loss.

DISCUSSION

There is a widely held belief that dietary factors are concerned in the development of human atherosclerosis, but this belief is not based on very tangible evidence. Rosenthal,[11] however, has compiled considerable data which indicate that a high rate of atherosclerosis is found among consumers of high-fat diets. An analogy between atheromatosis in cholesterol-fed rabbits and in man is hardly admissible since cholesterol feeding does not lead to marked, persistent hypercholesterolemia in man according to Hueper,[10] who has summarized the abundant, somewhat contradictory literature on this subject. While in a few instances persistent hyperlipemia or hypercholesterolemia is apparently associated with atherosclerosis in man, as in diabetes mellitus, multiple xanthomatosis, myxedema, and lipid nephrosis, in the large majority of cases such an association has not been demonstrated. In any event, dietary factors probably are not primarily concerned in any of these hyperlipemic states.

The state of nutrition in health and to a large extent in wasting disease is dependent on food consumption. The results of the present study indicate that the general state of nutrition is a factor not only in the development but also in the resolution of human atherosclerotic lesions. It may be inferred, therefore, that a dietary influence not necessarily associated with persistent hyperlipemia is involved in the pathogenesis of human atherosclerosis.

Since obesity is not invariably associated with severe atherosclerosis, and terminal weight loss is not always followed by appreciable resorp-

tion of lipid deposits, the state of nutrition may be only a secondary and subsidiary factor that becomes effective only if more fundamental, predisposing conditions are fulfilled; or only specific constituents in the diet may be concerned. For example, overnutrition due to high-fat or high-cholesterol diets may lead to atherosclerosis more readily than overnutrition due to high carbohydrate intake. The infrequent association of obesity and atherosclerosis in young persons and, in older age groups, the less constant association of the two in women than in men [1] argue against the explanation that specific items of food consumption are primarily involved. It is conceivable that both non-dietary, predisposing factors and the consumption of specific food materials in abundance are necessary to promote the development of atherosclerotic lesions.

It is frequently assumed that atherosclerosis is a slowly evolving, continuously progressive process. The implication of the findings reported here is that lipid deposits may be withdrawn from arterial deposits in relatively short periods of time. By inference, it is equally logical to assume that during periods of rapid gain in weight new deposits of lipid may form with equal rapidity. The atherosclerotic process may well progress and recede rapidly and intermittently, at least during the early stages of its development. The amount of lipid found in the intima at the end-stage may represent only a minute fraction of the total amount of lipid that has penetrated the vessel wall. The not uncommon discrepancies in evidence of generalized atherosclerosis as observed clinically and the degree found at necropsy are partly explainable on the basis of probable fluctuations in the anatomical manifestations of the disease.

SUMMARY AND CONCLUSIONS

A high incidence of severe atherosclerosis is found in obese persons at necropsy. Severe or even moderate atherosclerotic change is seldom observed in those with protracted undernutrition. When the group with terminal weight loss is not omitted from the analysis, a correlation between the state of nutrition and degree of atherosclerosis, although much less pronounced, is still demonstrable. When the analysis is limited to the degree of hyalinization and calcification of intimal plaques, inclusion of the group with terminal wasting disease almost totally obscures the relationship between nutrition and these features of the atherosclerotic process.

Less severe degrees of atherosclerotic change are usually observed in the group with wasting disease than in those without terminal weight loss. It is inferred, therefore, that significant resorption of previously

formed atheromatous lesions may occur during periods of marked weight loss. The most conspicuous and consistent difference in the two groups is observed in the amount of lipid contained in the intimal lesions. In general, less lipid, and in particular, fewer lipid-containing phagocytes are demonstrable in the intimal lesions of those with terminal weight loss. Less constant and significant differences are observed in other features of the atherosclerotic process in the two groups. It is suggested, therefore, that the early lesions are the ones that are most susceptible to regression during periods of weight loss.

REFERENCES

1. Wilens, S. L. Bearing of general nutritional state on atherosclerosis. *Arch. Int. Med.*, 1947, 79, 129–147.

2. Krylov, D. Sur l'artériosclérose expérimentale de l'aorte. *Compt. rend. Soc. de·biol.*, 1916, 79, 397–399.

3. Anitschkow, N. Über die Rückbildungsvorgänge bei der experimentellen Atherosklerose. *Verhandl. d. deutsch. path. Gesellsch.*, 1928, 23, 473–478.

4. Krisch, H. Beitrag zur Histologie und Bedeutung der Virchowschen "fettigen Usur" der Aorta. *Virchows Arch. f. path. Anat.*, 1921, 230, 191–214.

5. Zinserling, W. D. Untersuchungen über Atherosklerose. 1. Über die Aortaverfettung bei Kindern. *Virchows Arch. f. path. Anat.*, 1925, 255, 677–705.

6. Aschoff, L. Introduction. In: Cowdry, E. V. Arteriosclerosis. The Macmillan Co., New York, 1933, pp. 1–18.

7. Ophüls, W. The Pathogenesis of Arteriosclerosis. In: Cowdry, E. V. Arteriosclerosis. The Macmillan Co., New York, 1933, pp. 249–270.

8. Ribbert, H. Die Arteriosklerose. *Deutsche med. Wchnschr.*, 1918, 44, 953–957.

9. Leary, T. Cholesterol lysis in atheroma. *Arch. Path.*, 1944, 37, 16–19.

10. Hueper, W. C. Arteriosclerosis. *Arch. Path.*, 1945, 39, 117–131; 187–216.

11. Rosenthal, S. R. Studies in atherosclerosis: chemical, experimental and morphologic. I and II. *Arch. Path.*, 1934, 18, 473–506.

TWO SIMULTANEOUS CASES OF LEPROSY DEVELOPING IN TATTOOS *

Ross J. Porritt, M.D., and Richard E. Olsen, M.D.

(*From St. Joseph's Mercy Hospital, Pontiac, Mich.*)

Though leprosy is one of the oldest known diseases, there is still uncertainty and confusion concerning its transmission and the incubation period. Close association with lepers over a period of years has always been a recognized source of danger, and yet the few who developed leprosy, such as Father Damien, are notable exceptions. Minimal precautions seem adequate to prevent the spread of leprosy. Various theories as to transmission have enjoyed temporary popularity. Among the more intelligible of these were the concept of fish as intermediate hosts, the idea that wearing clothing discarded by lepers produced the disease, that person to person vaccination spread leprosy, that various insects acted as vectors, that sexual intercourse was responsible, and that leprosy was acquired through the nasal mucous membrane.

During the past 100 years, one of the most debated questions has been whether or not leprosy can be spread by the inoculation of tissue or other contaminated material from a leper into the skin of an uninfected person.

Jeanselme,[1] in 1934, concluded that there was no adequate proof of the transmission of leprosy by inoculation. He cited the experiments of Danielssen and Boeck,[2] Profeta,[3] and Mouritz [4] during the 19th century. Danielssen repeatedly inoculated himself but never showed evidence of leprosy. Profeta inoculated 10 persons experimentally without reproducing the disease. Mouritz likewise got negative results.

Klingmüller,[5] in 1930, gave a good review of the evidence for and against the experimental inoculation of leprosy.

Rogers and Muir,[6] in 1940, specifically accepted the transmission of leprosy by inoculation and considered it an important factor. They described, but questioned, the case of Keanu who was inoculated by Arning,[7] in 1886, in the Hawaiian Islands. He subsequently developed leprosy and died. Unfortunately, Keanu came from a leprous family and also lived in close contact with lepers. Cases accepted as valid evidence of leprosy developing after inoculation include that of Marchoux [8] who, in 1922, while operating on a leper, pricked the finger of his assistant who developed leprosy after several years. De Langen,[9] in 1931, reported the accidental inoculation of a patient by a physician who confused his syringes after giving a hypodermic to a leper. A leprous nodule developed at the site of the injection in 6 months. La-

goudaky,[10] in 1936, was repeatedly injected with blood from a leper. In less than 2 months after his first inoculation he developed cutaneous lesions of leprosy.

Negative results from experimental inoculations are difficult to evaluate because of the long incubation period and the possibility of subclinical or dormant infections as in tuberculosis. There is a wide variation of susceptibility to the disease among persons, between the sexes, in different age groups, and among races. Since leprosy tends to localize in scars and tattoos, and to be activated by trauma, it is difficult to prove that an infection following inoculation actually resulted from it.

The remarkable coincidences in the 2 cases to be presented offer very strong evidence favoring the transmission of leprosy by inoculation. Both men were members of the same unit in the United States Marine Corps. They were tattooed successively by the same man in Melbourne, Australia, on the same day in June, 1943. Both developed maculo-anesthetic leprosy in the tattoos about 2½ years later. Both the Marines and the tattooer were inebriated and a number of needles were broken during the process. One man had multiple tattoos on his left arm but developed leprosy only in the one made in Melbourne. The two men in civil life are friends and residents of the same town, and one was instrumental in bringing the other to the doctor for diagnosis. A third man tattooed at the same place, but at a different time, as yet shows no evidence of leprosy.

Case 1

The patient, W. J. H., was a white male, 24 years of age, whose chief complaint was that of increased pigmentation and numbness of a tattoo on the back of the left forearm.

Present Illness. In June, 1943, the patient, while in company with L. G. (case 2), was tattooed on the extensor surface of the lower left forearm in Melbourne, Australia. Nothing took place to suggest any untoward development in the tattoo until March or April of 1946 when the patient noticed that the area of the tattoo and a zone about 1.5 cm. in width about it had become pale red (Fig. 1), and was insensitive to light touch and pain. During June, 1946, while at a party, he deliberately burned himself in this lesion with a lighted cigarette to prove the loss of sensation.

Past Illness. The patient had had the usual childhood diseases. He had developed malaria in December, 1942. The left scapula had been chipped in an accident during September, 1945, and he has noted some weakness in his left arm since that time.

Family History. The family history was not significant.

Physical Examination. The patient's temperature was 98.6°F.; pulse, 76 per minute; blood pressure, 100/60 mm. Hg. On routine physical examination a slight congestion of the nasal mucous membrane was noted and the left upper arm was found to be 8 mm. less in circumference than the right arm. The axillary and inguinal lymph nodes were small. There was a nontender swelling on the lateral surface of the juncture of the middle and lower thirds of the left upper arm. This

measured about 0.5 by 4 cm. There was no palpable evidence of lesions of the nerve trunks. The reflexes were normal. There were two tattoos on the extensor surface of the left forearm. About the distal one (made in Melbourne, as previously noted) there was a uniform, pale fawn-colored area involving the entire tattoo and a narrow zone about it. The total diameter was 9 cm. There was a loss of sensation to pain and light touch in the discolored area. There were three small depressed scars in the center, each about 0.5 cm. in diameter. The other tattoo showed no evidence of disease.

Laboratory Examination. Laboratory findings were as follows: The Kahn reaction of the blood was negative. Hemoglobin was 16 gm.; red blood cell count, 4,630,000; white blood cell count, 6,300; differential count, 46 per cent polynuclear neutrophils with 37 per cent segmented cells, 7 per cent stab cells, and 2 per cent juveniles, 7 per cent large and 45 per cent small lymphocytes, 8 per cent eosinophils, and 1 per cent monocytes. Routine chemical and microscopic urinalyses were normal.

On November 2, 1946, tissue was taken for biopsy from the pigmented area outside the tattoo. The excision was made without benefit of anesthetic and caused the patient no discomfort. The microscopic sections showed a tuberculoid reaction, and occasional acid-fast bacilli, averaging about four per section, were found by the Ziehl-Neelsen method. Smears from the nasal mucous membrane were negative for acid-fast bacilli. Intradermal inoculation with 0.1 cc. of O.T. (1: 10,000) was positive. In view of the history and microscopic findings, a provisional diagnosis of cutaneous tuberculoid leprosy was made.

Case 2

L. G. was a white male, 25 years old, whose chief complaint was numbness and pigmentation of a tattoo on the flexor surface of the left forearm.

Present Illness. In June, 1943, the patient, in company with W. J. H. (case 1), was tattooed on the flexor surface of the left forearm in Melbourne, Australia. About January, 1946, he noticed that the area of the tattoo and a zone about it 1.5 cm. in width about it was becoming dusky red (Fig. 2) and numb. Since then the color had gradually darkened. Two new areas (Fig. 3) had appeared over the triceps of the upper left arm 7.5 cm. above the elbow. These became confluent. They were a dark violaceous color and were numb. There was no elevation of the skin surface. The patient's general health remained good.

Past Illness. The patient had had the usual childhood diseases and had developed malaria in December, 1942. He had had occasional malarial chills since.

Family History. The family history was not pertinent.

Physical Examination. The patient's temperature was 99°F.; the pulse rate, 72 per minute; blood pressure, 120/70 mm. Hg. Routine examination of the head, neck, chest, abdomen, and genitalia showed no abnormality. No surgical scars were present. In the left lumbar region at the waistline there was a violaceous, flat lesion 1 cm. in diameter. This had normal sensation and was not definitely related to the present illness. The axillary and inguinal lymph nodes were normal in size. On the flexor surface of the left forearm there was a tattoo. The skin of the entire tattoo and a zone 1.5 cm. in width about it showed a violaceous discoloration. There was a loss of sensation of pain and to light touch throughout the entire pigmented area. There was no ulceration or elevation present. On the extensor surface of the left upper arm 7.5 cm. above the elbow there were two coalescent lesions making an hour-glass-shaped area about 2.5 by 4 cm. This had the same

violaceous color as the tattooed area and there was the same loss of sensation. There was no palpable abnormality of the nerve trunks.

Laboratory Examination. Laboratory findings were as follows: The Kahn reaction of the blood was negative; hemoglobin was 18.6 gm.; red blood cell count, 5,110,000; white blood cell count 6,500; differential count, 45 per cent polynuclear neutrophils with 41 per cent segmented cells, 3 per cent stab cells, and 1 per cent juveniles, 7 per cent large and 32 per cent small lymphocytes, 14 per cent eosinophils, and 2 per cent monocytes. The routine chemical and microscopic urinalyses were normal.

On November 11, 1946, a specimen was taken for biopsy from the pigmented area outside the tattoo. No anesthetic was necessary due to the lack of sensation of pain. The tissue was divided into two parts. One was sent in saline solution to the Michigan Department of Health. The report received stated that no acid-fast bacilli were found in direct smears or in culture. Two guinea-pigs inoculated with the tissue taken for biopsy showed no evidence of tuberculosis after 7 weeks.

The other part of the tissue was fixed in formalin and embedded in paraffin. The microscopic appearance was the same as in case 1. Acid-fast bacilli were demonstrated by the Ziehl-Neelsen method, but they were less common than in case 1 and averaged only about one per section. Smears from the nasal mucous membrane were negative for acid-fast bacilli. Intradermal inoculation with 0.1 cc. of O.T. (1: 10,000) was negative. The tentative diagnosis was cutaneous tuberculoid leprosy.

The first case was informally described to Dr. Claude Behn of Detroit, who, without seeing the patient, made the original suggestion of leprosy as a probable diagnosis.

Unstained sections from both cases were submitted to the United States Public Health Service. From the U.S. Marine Hospital (Leprosarium) at Carville, Louisiana, came an unequivocal diagnosis of "typical tuberculoid leprosy," but acid-fast bacilli were not demonstrated.

These lesions could well be tuberculous as far as the gross appearance is concerned. Microscopically, the presence of epithelioid tubercles with Langhans' giant cells, lymphocytic and plasma cell infiltration, and occasional acid-fast bacilli are as characteristic of tuberculosis as of leprosy. The history is more suggestive of leprosy than tuberculosis. The loss of sensation to pain and light touch in the pigmented lesions, the presence of a positive tuberculin skin test in case 1 and a negative test in case 2, the failure of guinea-pigs to develop tuberculosis after inoculation with tissue containing the acid-fast bacilli, the failure to culture acid-fast bacilli, the presence of vacuolated cells, and the positive diagnosis received from the U.S. Marine Hospital at

Carville, Louisiana, establish these as cases of maculo-anesthetic or tuberculoid leprosy.

The long incubation period suggests resistance to the disease on the part of the patients. The extensive traumatization of the skin incident to tattooing might favor the development of the disease. It is possible that the multiple skin punctures led to massive inoculation, but that is purely speculative.

It is of interest that, as has been noted by other observers, cinnabar (red mercuric sulphide) in the tattoos, which discourages spirochetal activity in syphilis of the skin, does not have any apparent effect on the bacillus of leprosy.

The tissues taken for biopsy from the two patients were so similar that a single description will suffice. In each case the specimen was taken from the pigmented lesion, near its edge, outside the tattoo. Gross examination of the tissue showed a smooth skin surface with moderate pigmentation and no ulceration. The microscopic appearance (Figs. 4 to 8) of the tissue so closely resembled tuberculosis of the skin as to be almost, if not quite, indistinguishable. Many of the tubercles suggested Boeck's sarcoid but occasional ones showed an appreciable degree of caseous necrosis. The Langhans' giant cells were of all sizes and their appearance and distribution were no different from those of tuberculosis. They occurred both in the epithelioid foci and scattered through the areas of lymphocytic infiltration. The characteristic lesion was tuberculoid in type. It consisted of a center of epithelioid cells with a rim of lymphocytes, a few plasma cells, and even fewer polymorphonuclear cells. Occasional eosinophilic leukocytes were present, at times infiltrating between the epithelioid cells. The nodule was largely avascular. The largest showed some central caseous necrosis but this was not common. The tuberculoid foci were present throughout the tissue specimen, both in the corium and subcutaneous fat. The process apparently extended beyond the depth of the excised tissue.

The epidermis was irregularly atrophic and there was flattening and partial loss of the dermal papillae. In some areas there was lymphocytic invasion of the basal and prickle cell layers. There was no tendency to epithelial overgrowth, such as occurs in blastomycosis.

The hair follicles had lymphocytic infiltration about and in them and the picture was entirely compatible with the loss of hair characteristic of lepromas. There was a granulomatous involvement of the sweat glands, some of which had almost completely disappeared, being replaced by epithelioid nodules with lymphocytes and plasma cells.

The largest tuberculoid foci were present in the deep layer of the

corium, with smaller nodules and extensive lymphocytic infiltration in the superficial layer. There were no characteristic leprous foam cells, which usually contain large numbers of acid-fast bacilli, but there were occasional vacuolated cells which were suggestive.

The tuberculoid foci in the subcutaneous fat were more discrete than those nearer the epithelial surface and there was no generalized involvement of the adipose tissue. Here the tubercles were smaller than those in the deep layer of the corium.

The cutaneous nerves were involved but not more so than other structures. There was no particular evidence in these tissues that the process was extending by way of the nerves. There was quite extensive involvement of the small vessels but they did not show the swelling and proliferation of the endothelium which is found in syphilis.

Ziehl-Neelsen staining of the sections showed occasional acid-fast bacilli in the first case and rare ones in the second. The bacilli were found most often in or about the largest tuberculoid lesions in the deep layer of the corium. Usually they occurred in pairs or with two single organisms in one oil-immersion field. The acid-fast bacilli showed no significant variation from tubercle bacilli either in morphologic characteristics or staining qualities.

SUMMARY

Two men from the same community, while serving in the United States Marine Corps, were tattooed by the same man on the same day in June, 1943, at Melbourne, Australia. They both developed maculoanesthetic or tuberculoid leprosy in their tattoos during the first half of 1946. One man had multiple tattoos but developed leprosy only in the tattoo made in Melbourne the day when his friend was tattooed. A third Marine, tattooed at the same place but not on the same day, has shown no evidence of leprosy. These two cases provide strong evidence for the spread of leprosy by inoculation.

REFERENCES

1. Jeanselme, E. La lèpre. G. Doin et Cie., Paris, 1934, p. 214. (Cited from Lagoudaky.[10])
2. Danielssen, D. C., and Boeck, W. Traité de la spédalskhed ou éléphantiasis des Grecs. J. B. Baillière, Paris, 1848. (Cited from Klingmüller.[5])
3. Profeta, G. Sulla elefantiasi dei Greci. *Gior. internaz. d. sc. med.,* 1884, 6, 282–299.
4. Mouritz, A. A. St. M. "The Path of the Destroyer." A History of Leprosy in the Hawaiian Islands and Thirty Years Research into the Means by Which It Has Been Spread. Honolulu Star Bulletin, Ltd., Honolulu, 1916, pp. 140–151.

5. Klingmüller, V. Die Lepra. Handbuch der Haut- und Geschlechtskrankheiten. J. Springer, Berlin, 1930, 10, Pt. 2, 131–134.

6. Rogers, L., and Muir, E. Leprosy. J. Wright & Sons, Ltd., Bristol, 1940, ed. 2, pp. 92–94.

7. Arning, E. Appendix to the Report on Leprosy of the President of the Board of Health to the Legislative Assembly of 1886. P. C. Advertiser Steam Print, Honolulu, 1886, Appendix I, xxxvii-liv.

8. Marchoux, E. Un cas d'inoculation accidentelle du bacille de Hansen en pays non lepreux. *Internat. J. Leprosy,* 1934, 2, 1–6.

9. de Langen, C. D. Superinfection with leprosy. Transactions of the Eighth (Bangkok) Congress of the Far Eastern Association of Tropical Medicine, 1931, 2, 499. (Cited from a condensation in: *Internat. J. Leprosy,* 1933, 1, 220–225.)

10. Lagoudaky, S. Preliminary note on self-inoculation of leprosy. *J. Trop. Med.,* 1936, 39, 81–83. Self-inoculation of leprosy—second communication. *Ibid.,* 1937, 40, 77–79.

[Illustrations follow]

DESCRIPTION OF PLATES

PLATE 127

FIG. 1. Case 1. The smaller distal tattoo made in Melbourne is the only one with pigmentation and anesthesia. The pigmentation is so light that it does not appear in the photograph. The dark spot by the star indicates the site where tissue was excised for biopsy.

FIG. 2. Case 2. Tattoo on the left forearm showing the extent of the pigmentation. The skin suture is still present where tissue was taken for biopsy.

FIG. 3. Case 2. Secondary lesions on the extensor surface of the left upper arm.

FIG. 4. Case 2. Large Langhans' giant cell and a small epithelioid tubercle with lymphocytic infiltration about them. Hematoxylin and eosin stain. × 500.

Porritt and Olsen

Leprosy Developing in Tattoos
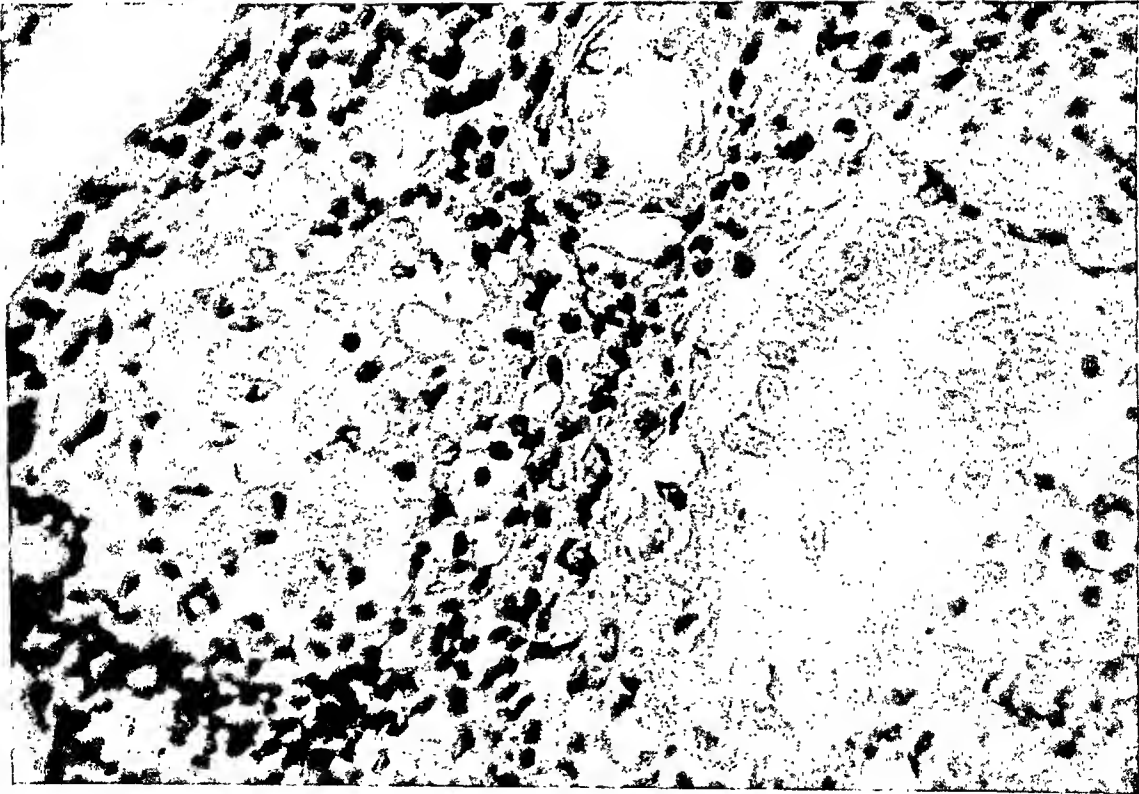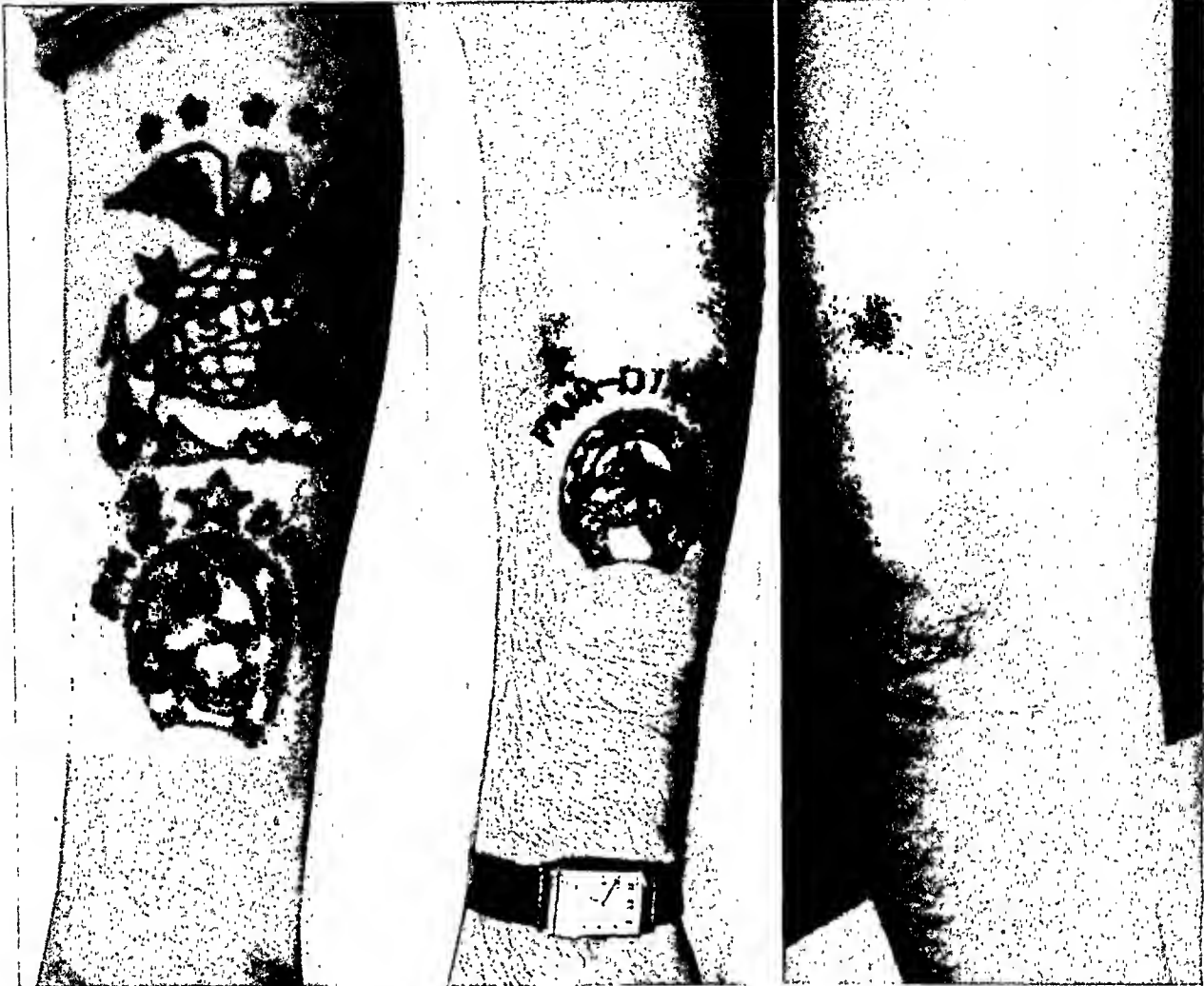
PLATE 128

FIG. 5. Case 1. Lepromatous reaction about a sweat gland. Hematoxylin and eosin stain. X 500.

FIG. 6. Case 1. Lymphocytic infiltration of the epithelium and corium with a well formed Langhans' giant cell. Hematoxylin and eosin stain. X 500.

Porritt and Olsen

Leprosy Developing in Tattoos
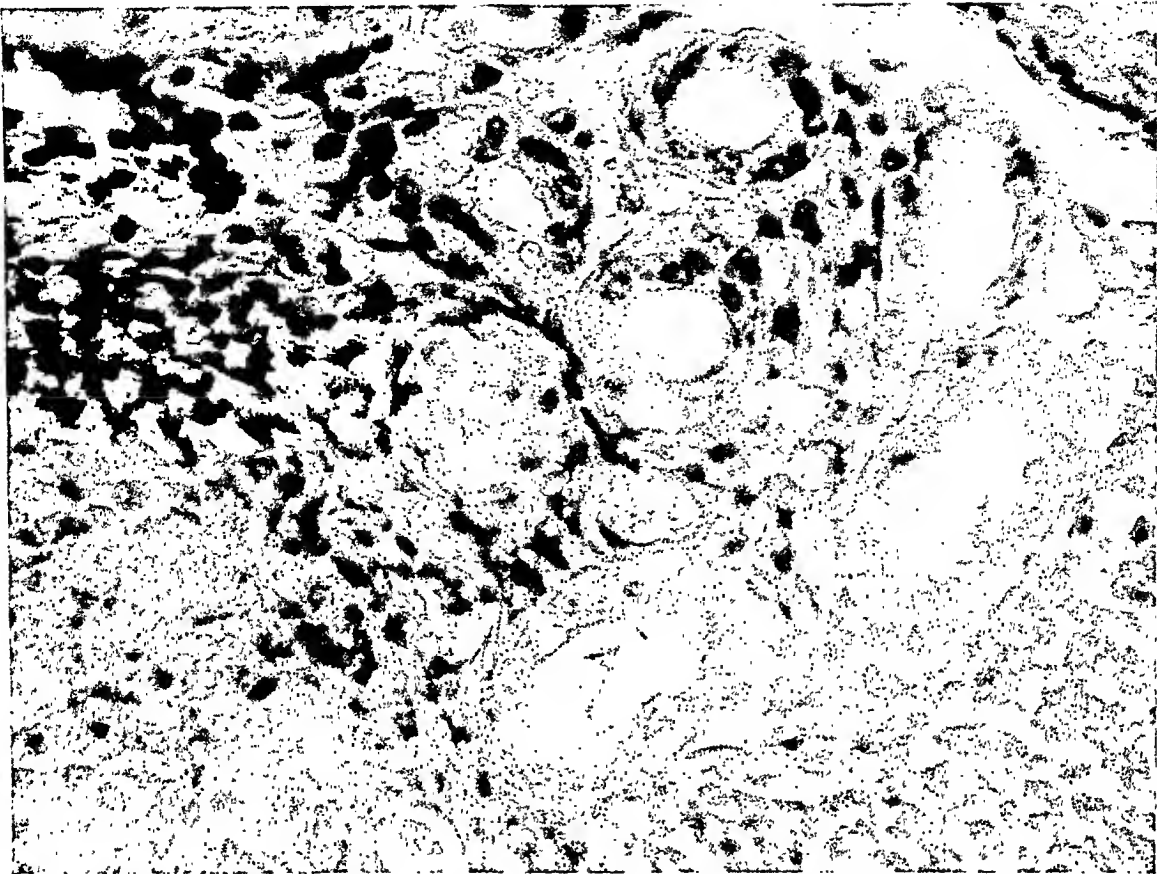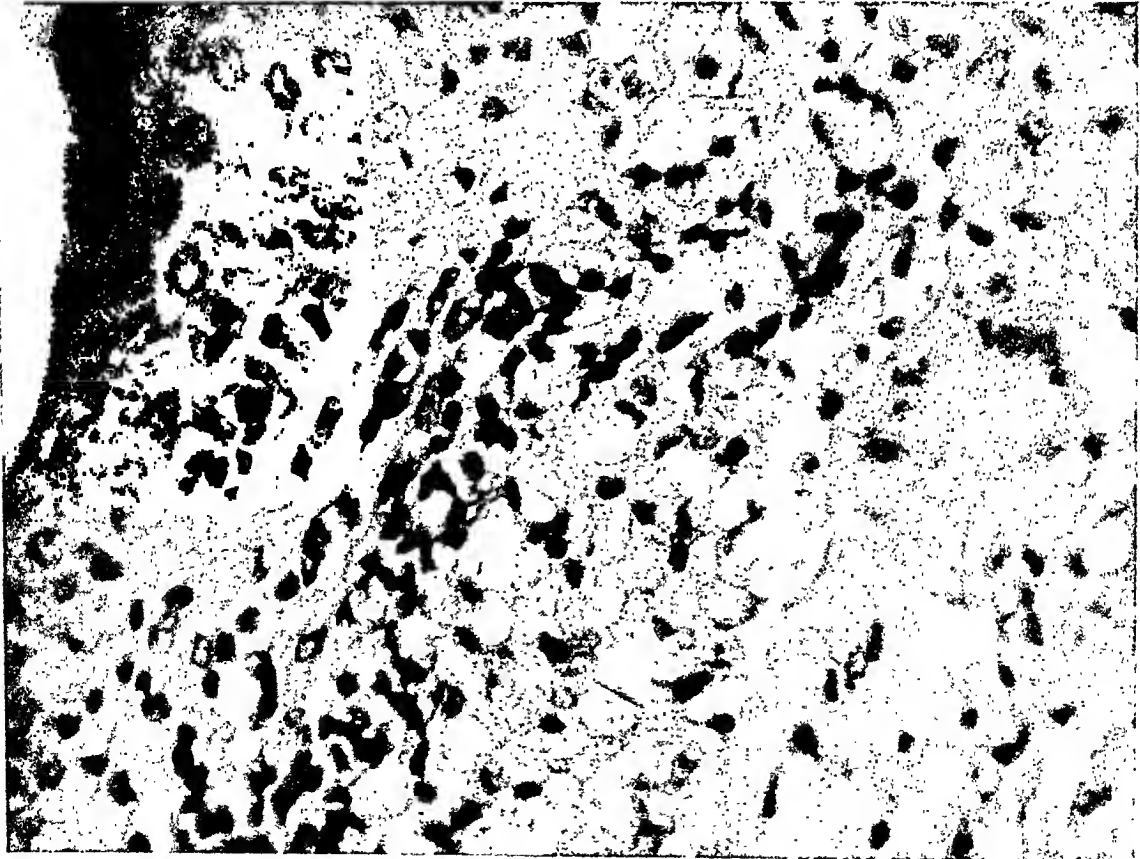
PLATE 129

FIG. 7. Case 1. Lymphocytic infiltration in and about a hair follicle. Hematoxylin and eosin stain. $\times$ 500.

FIG. 8. Case 2. Large epithelioid tubercle with caseous necrosis in the center. This lesion is microscopically indistinguishable from tuberculosis. Hematoxylin and eosin stain. $\times$ 180.

Porritt and Olsen
Leprosy Developing in Tattoos
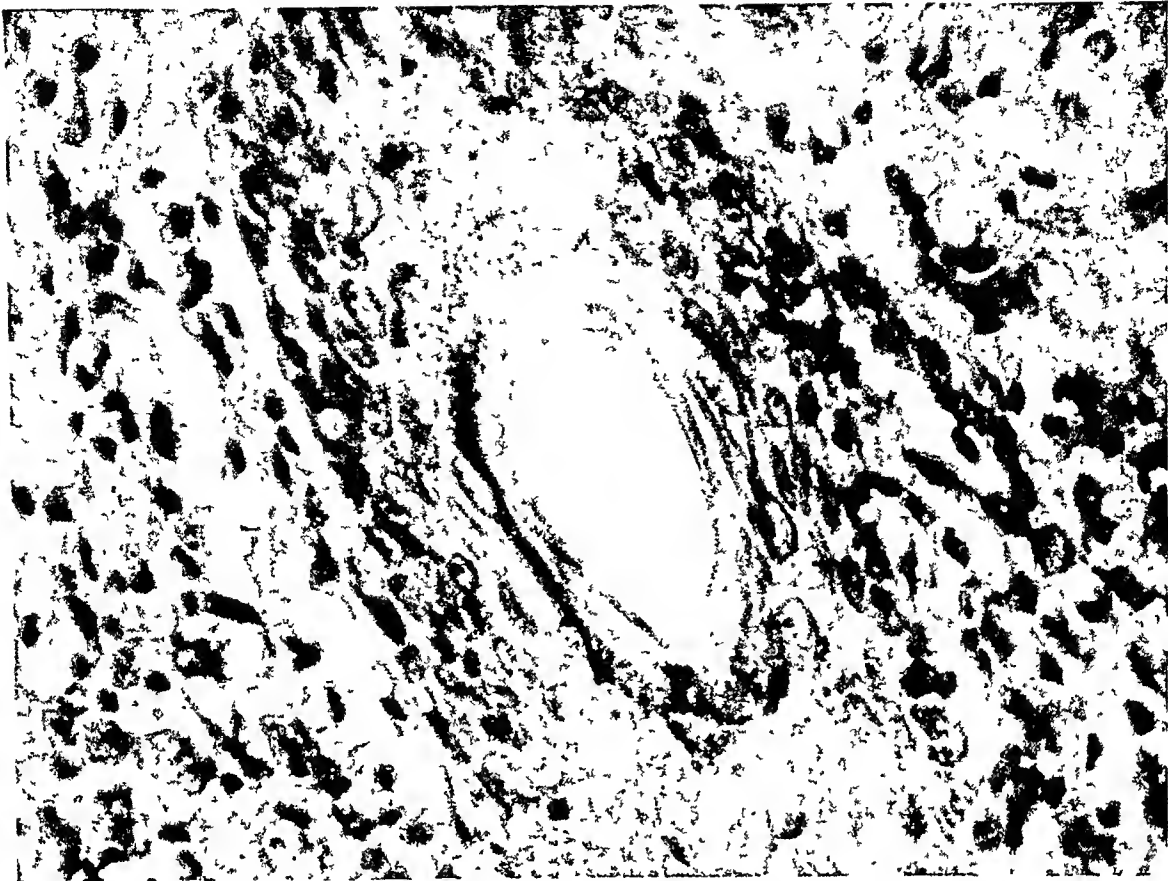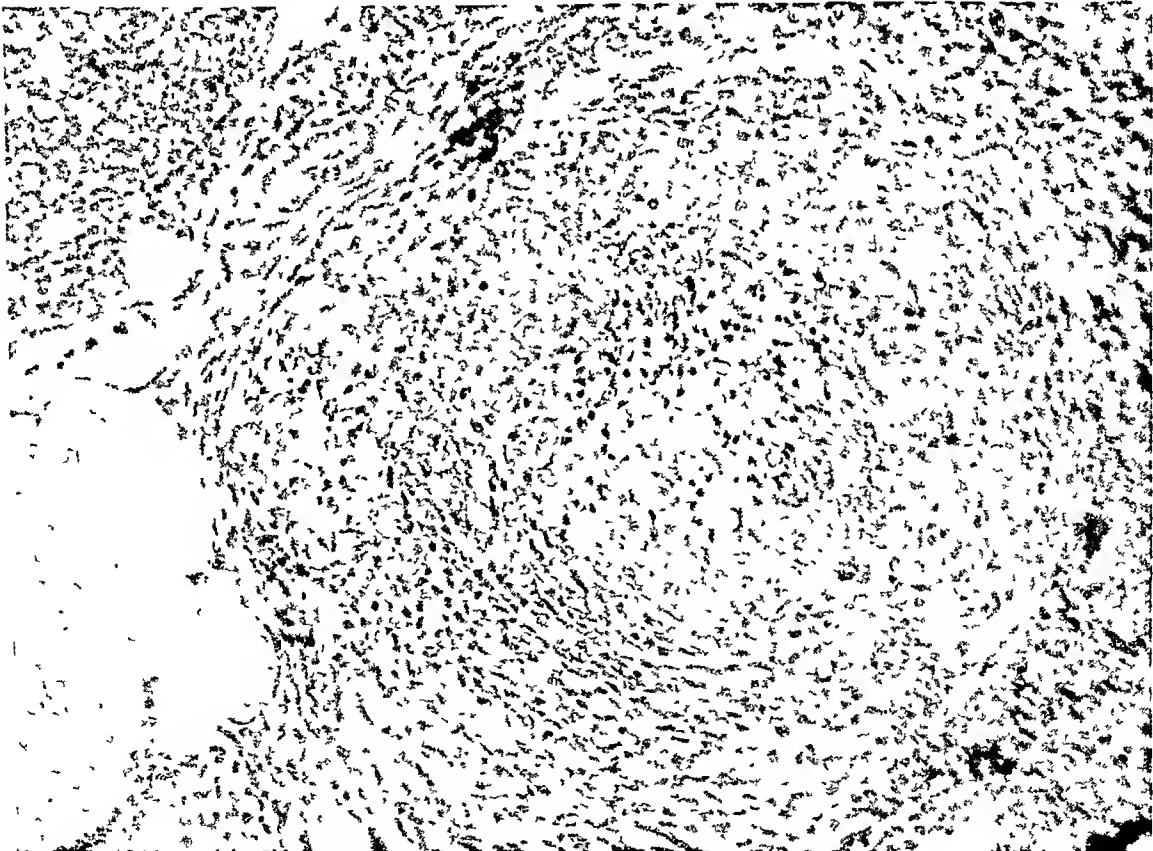
THE INTESTINAL PHASE OF HUMAN TRICHINOSIS *

WALTER A. STRYKER, M.D.†

(*From the Department of Pathology, University of Michigan, Ann Arbor, Mich.*)

Study of the tissues of fatal cases of trichinosis in man has been concerned largely with the lesions in muscle, heart, and brain produced by the larval form of the parasite. Discovery of adult worms in the intestine has been reported in only a few instances,[1-7] and in some of these reports this observation has not been confirmed by microscopic examination. The presence of gravid females is of major clinical importance since from them come the larvae which cause the muscular, cardiac, and cerebral lesions. Determination of the maximal duration of the intestinal phase in the human host can be made only by observations upon human material, since in each species the factors influencing the duration are different. Even within a species the duration of the intestinal phase will be influenced by such factors as the extent (heaviness) of the infestation and the prior contact of the host with the parasite.

The duration of productivity of the adult female trichina is generally stated to be about 6 weeks. This figure is based chiefly on experimental work with animals, especially that of Roth [8] with guineapigs. In these few reported instances in which the presence of adult worms in the human intestine has been confirmed by microscopic examination, the longest duration of the infection was 30 days [6]; in one report [9] of a patient dying on the 56th day of illness it is stated that no parasites could be found in the intestine.

REPORT OF CASE

In the necropsy service of the Department of Pathology of the University of Michigan a case of trichinosis has been observed in which many living adult worms were demonstrable in the intestine. The patient was a tavern keeper, 35 years old, who had eaten raw pork sausage prepared from a hog he had raised and butchered. Other members of his family and neighbors who had eaten the raw meat likewise developed trichinosis, and the disease proved fatal also to an 8-year-old daughter. It was stated positively by both the patient's wife and the family physician that the raw sausage had been consumed on only one occasion. The father and the daughter ate large amounts of the meat. The clinical course of the father was characterized by diarrhea, marked muscular pain, fever, and terminal respiratory distress. There were

physical signs and electrocardiographic findings indicating myocardial damage and neurologic abnormalities suggesting cerebral damage. The patient was hospitalized for the last 4 weeks of life. Death occurred on the 54th day after the eating of the raw pork and sausage.

The gross examination of the intestines at the time of autopsy revealed only scattered petechial hemorrhages in the mucosa. No parasites were seen. A portion of the bowel was fixed unopened so as to preserve the contents.

On microscopic examination, adult trichinae were found in many sections of both the large and small intestine; they were most numerous in the jejunum. Both male and female worms were present. In the latter, larval forms could usually be recognized. Adult worms were more numerous in the sections made from unopened intestine, where the contents of the lumen were undisturbed. In single sections the trichinae could be found lying free in the lumen of the intestine (Fig. 1) or embedded in the mucosa (Fig. 2). When traced by serial sections a worm was frequently found to have one end or its mid-portion embedded in the mucosa, while the remainder extended into the lumen. In some areas the mucosa completely enclosed a portion of the adult worm. Most frequently it was the uterine area that was embedded in, or in contact with, the mucosa. Occasionally a larval form could be found lying in the mucosa adjacent to an adult. There was no significant cellular reaction to the parasites within the mucosa. Lymphocytes, plasma cells, and eosinophils were found near worms; but such cells were present equally in the mucosa distant from the embedded parasites and in control sections from other cases in which no parasites were found. There were no areas of granulomatous inflammation and no foci of necrosis or hemorrhage. The mucosal glands and connective tissue near a worm were compressed by it; these elements were directly in contact with the cuticle with no surrounding area of lysis. One adult trichina was found to have penetrated the mucosa to the level of the muscularis mucosae. No adult worms or larvae were found external to the muscularis mucosae.

Sections of skeletal muscle from many areas were examined and in all was an unusually heavy parasitization by larval trichinae (Fig. 3). Even in the fibers of the cremaster muscle along the spermatic cord many larvae were found. Most of the larvae were coiled and formation of the wall of the cyst had begun. A few larvae were still straight. Single fibers contained as many as three larvae at the level of a single section. Many muscle fibers showed hyaline degeneration, and there was a heavy cellular infiltration between the muscle fibers; the infiltration was composed of mononuclear cells of macrophagic type, lympho-

cytes, plasma cells, and many eosinophils. In a few foci the exudate was purulent. Quantitative examination of the diaphragm for content of trichinae was performed by Dr. S. E. Gould.* Each gram of diaphragmatic muscle from near its tendinous attachment, examined by the digestion method, revealed an average of 2677 larvae. This is one of the heaviest infestations that has been reported. Examination of the heart and the brain revealed typical trichinous myocarditis [10] and encephalitis.[5, 11] Larvae were demonstrable in each of these organs (Fig. 4).

DISCUSSION

The length of time living adult trichinae remain in the intestinal canal is related to three factors: the species of animal parasitized, the state of immunity present in the host, and the heaviness of the infestation. These three factors are closely correlated, and in each case all are effective.

Trichinella spiralis is able to parasitize a wide range of hosts, chiefly mammalian. There is a strong correlation between the natural resistance of the host to the lethal effects of generalized trichinosis (natural immunity) and the length of time the worms remain in the intestine. This is exhibited by a reciprocal relationship. The more tolerant or resistant the host to the effects of larval trichinae, the shorter the time that living adults can remain in the intestine. The dog has been found to be relatively resistant to infection; live adult worms have been seen in the canine intestine for only 10 days after feeding.[8] In the gopher, relatively susceptible to the parasite, intestinal trichinae have been found as long as 13 weeks after infection.[12]

The intestinal phase of the life cycle of *T. spiralis* has also been demonstrated to be related to acquired immunity. Immunity against re-infection by the parasite was shown by Ducas [13] to be localized in the intestines, and this conclusion has been confirmed in numerous subsequent investigations.[14-21] The mechanism of acquired immunity depends apparently on retardation of the development of ingested larvae to adult worms, plus a deleterious effect upon any adult worms present.[14, 18, 22] In immune rats there has been observed a rapid loss of larvae from the intestines 8 to 18 hours after feeding.[23]

A relation between the number of infecting larvae and the time during which adults could be found in the intestine of rats was shown by McCoy.[24] In light infections the adults could be recovered for only 14 to 16 days. With heavier infections, adults could be found for 4 to 5 weeks. With massive infection, death resulted in 2 weeks from the effects of the intestinal phase. In the guinea-pig, Roth[8] was not able

* Wayne County General Hospital, Eloise, Michigan.

to demonstrate a correlation between infecting dose and duration of the intestinal phase.

The results of animal experimentation lead to conclusions which are applicable to observations of human trichinosis. Man is relatively susceptible to trichinosis, and thus the intestinal phase may be prolonged. In the first contact of the host with the parasite acquired immunity does not develop, and this also tends to cause a prolonged intestinal phase. In a second infection in man, the adults should have a shorter life. The number of larvae ingested is relatively small in most persons. The "dilution" of pork from an infected pig by combining it with meat from noninfected animals increases the number of persons infected but decreases the extent of the infection in each individual. However, when an infected animal is the sole source of meat, the infection may be heavy and a long intestinal phase may result.

In the case here reported, the infection was a first infection (all muscular lesions were recent) and the quantity of larvae ingested was great. Thus the long intestinal phase in this patient is in accord with the conclusions obtained from animal experimentation.

The significance of a prolonged intestinal phase in trichinosis is obvious. Persistence of systemic myositis and increased severity and duration of the characteristic myocarditis and encephalitis result. Death during the acute phase of trichinosis is usually due to the lesions of the heart and central nervous system. Pulmonary embolism and infarction frequently seen in fatal trichinosis may also be related to the number of larvae, in that immobilization by the pain of muscular movements favors hemostasis with resulting thrombosis and embolism. The heart and the brain are affected only while the larvae are in a migratory state, since no encystment occurs in these organs. With disappearance of the larvae the inflammation subsides. These facts were clearly illustrated in the case here presented. Well formed larvae were demonstrable in both the heart and the brain, and there was granulomatous inflammation of these organs. The clinical course included symptoms indicating both myocarditis and encephalitis. The discovery of adult worms in the intestine and the presence of larvae within adult females showed that release of larvae was still occurring at the time of the patient's death, 54 days after eating the infected meat.

These observations emphasize the importance of therapeutic efforts directed against the adult trichinae in the intestine, either by the use of purgatives or of immune or convalescent serum. There is evidence that many of the adults can be removed by purgation.[25, 26] Immune or convalescent serum is believed to act upon the ingested larvae which

are maturing in the intestines and thus decrease the number of effective adults.[22] There is no evidence that anti-helminthic drugs are of value.[27] Purgation, with careful attention to fluid balance and the general state of the patient, at present offers the most hope for reducing the number of gravid female worms.

SUMMARY

Living adult trichinae, including gravid females, were demonstrated in the intestine of a fatal case of human trichinosis 54 days after ingestion of infected pork. This is the longest period of persistence of adult trichinae in the human intestine thus far reported, with microscopic demonstration of the adult parasites *in situ*. The possibility of continued release of larvae over a period of even greater length must be taken into account in the therapeutic management of trichinosis.

REFERENCES

1. Zenker, F. A. Ueber die Trichinen-Krankheit des Menschen. *Virchows Arch. f. path. Anat.*, 1860, 18, 561–572.
2. Cohnheim, J. Zur pathologischen Anatomie der Trichinenkrankheit. *Virchows Arch. f. path. Anat.*, 1866, 36, 161–186.
3. Kratz, F. Die Trichinenepidemie zu Hedersleben. W. Engelmann, Leipzig, 1866. (Cited by Gould, S. E. Trichinosis. C. C. Thomas, Springfield, Ill., 1945, p. 79.)
4. Prym, P. Ueber Trichinose beim Menschen. *Centralbl. f. allg. Path. u. path. Anat.*, 1923–24, 34, 89–94.
5. Gruber, G. B. Über die Beteiligung des Herzens und der Gefässe an der menschlichen Trichinose. *Zentralbl. f. Herz- u. Gefässkr.*, 1925, 17, 319–327; 347–352; 359–367; 381–389; 399–406.
6. Horlick, S. S., and Becknell, R. E. Trichiniasis with widespread infestation of many tissues. *New England J. Med.*, 1929, 201, 816–819.
7. Terry, L. L., and Work, J. L. Trichinosis of the myocardium. Report of a case, with autopsy findings. *Am. Heart J.*, 1940, 19, 478–485.
8. Roth, H. On the localization of adult trichinae in the intestine. *J. Parasitol.*, 1938, 24, 225–231.
9. Pavlica, F. Zur intrauterinen Übertragung der Trichinose. *Med. Klin.*, 1927, 23, 1973.
10. Weller, C. V., and Shaw, M. Myocardial failure due to trichinosis. Preliminary report. *Tr. A. Am. Physicians*, 1932, 47, 41–46.
11. Gordon, M. B., Cares, R., and Kaufman, B. Encephalitis and myocarditis in a fatal case of trichinosis. Report of a case in a fourteen-year-old girl. *J. Pediat.*, 1935, 6, 667–675.
12. Offutt, E. P., Jr., and McCoy, O. R. The "gopher," *Citellus richardsonii* (Sabine), as an experimental host for *Trichinella spiralis*. *J. Parasitol.*, 1941, 27, 535–538.
13. Ducas, R. L'immunité dans la trichinose. Thèse. Jouve et Cie., Paris, 1921. (Cited by Gould, S. E. Trichinosis. C. C. Thomas, Springfield, Ill., 1945, p. 133.)
14. McCoy, O. R. Immunity of rats to re-infection with *Trichinella spiralis*. *Am. J. Hyg.*, 1931, 14, 484–494.

15. Bachman, G. W., and Rodríguez Molina, R. Resistance to infestation with Trichinella spiralis in hogs. The eosinophilic and precipitin response. *Am. J. Hyg.*, 1933, 18, 266–294.

16. McCoy, O. R. Artificial immunization of rats against Trichinella spiralis. *Am. J. Hyg.*, 1935, 21, 200–213.

17. Bachman, G. W., and Oliver González, J. Immunization in rats against Trichinella spiralis. *Proc. Soc. Exper. Biol. & Med.*, 1936, 35, 215–217.

18. Culbertson, J. T., and Kaplan, S. S. Passive immunity in experimental trichiniasis. (Abstract.) *J. Parasitol.*, 1937, 23, 560.

19. Roth, H. Immunitaetsverhaeltnisse bei experimenteller Trichinose. *Acta path. et microbiol. Scandinav.*, 1938, suppl. 38, 132–136.

20. Roth, H. Experimental studies on the course of trichina infection in guinea pigs. III. Immunity of guinea pigs to re-infection with Trichinella spiralis. *Am. J. Hyg.*, 1939, 30, Sect. D, 35–64.

21. Fischthal, J. H. Number of larvae and time required to produce active immunity in rats against Trichinella spiralis. *J. Parasitol.*, 1943, 29, 123–126.

22. Culbertson, J. T., and Kaplan, S. S. A study upon passive immunity in experimental trichiniasis. *Parasitology*, 1938, 30, 155–166.

23. McCoy, O. R. Rapid loss of Trichinella larvae fed to immune rats and its bearing on the mechanism of immunity. (Abstract.) *J. Parasitol.*, 1938, suppl. 24, 35.

24. McCoy, O. R. Size of infection as an influence on the persistence of adult trichinae in rats. *Science*, 1932, 75, 364–365.

25. Birch, C. L. Trichinosis from the barbecue stand. *M. Clin. North America*, 1931, 15, 791–796.

26. Fantus, B. The therapy of the Cook County Hospital: Therapy of trichiniasis. *J. A. M. A.*, 1935, 104, 473.

27. Gould, S. E. Trichinosis. C. C. Thomas, Springfield, Ill., 1945, pp. 266–274.

DESCRIPTION OF PLATES

PLATE 130

FIG. 1. Adult female *Trichinella spiralis* in small intestine. Larvae can be seen within the uterus. Hematoxylin and eosin stain. × 200.

FIG. 2. Cross section of an adult female *Trichinella spiralis* in the mucosa of the small intestine. Numerous larvae are present within the uterus. Hematoxylin and eosin stain. × 600.
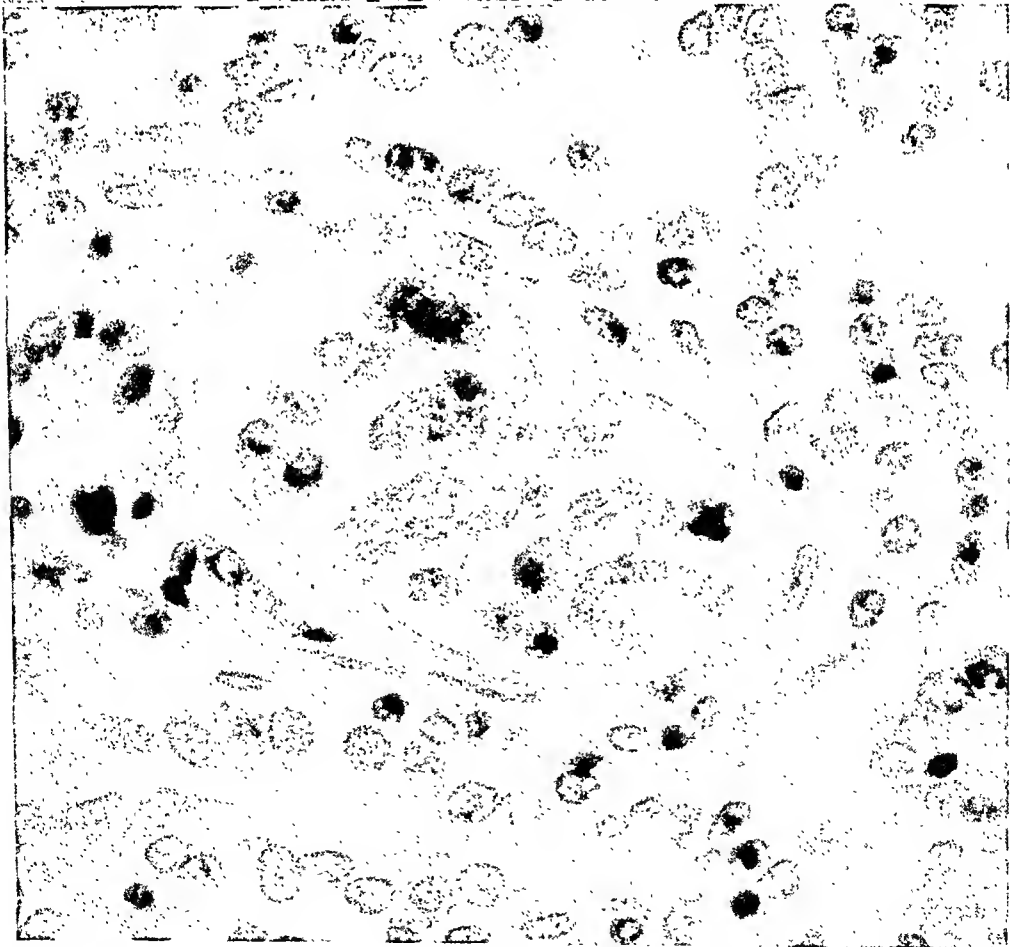
PLATE 131

FIG. 3. Encysted *Trichinella spiralis* in the diaphragm. Hematoxylin and eosin stain. $\times$ 150.

FIG. 4. Larval *Trichinella spiralis* in brain. The larva in upper center of field lies in a small granuloma. Hematoxylin and eosin stain. $\times$ 800.

3

4

Stryker Intestinal Phase of Human Trichinosis
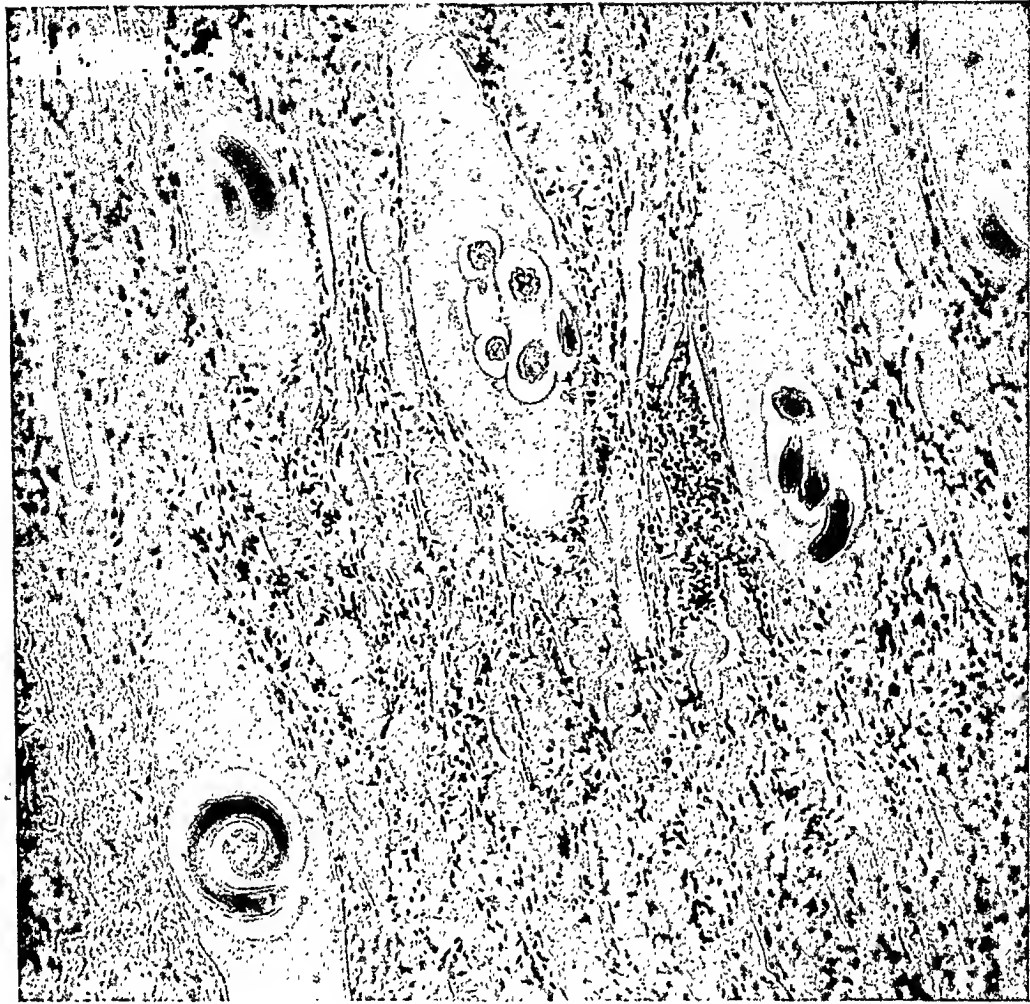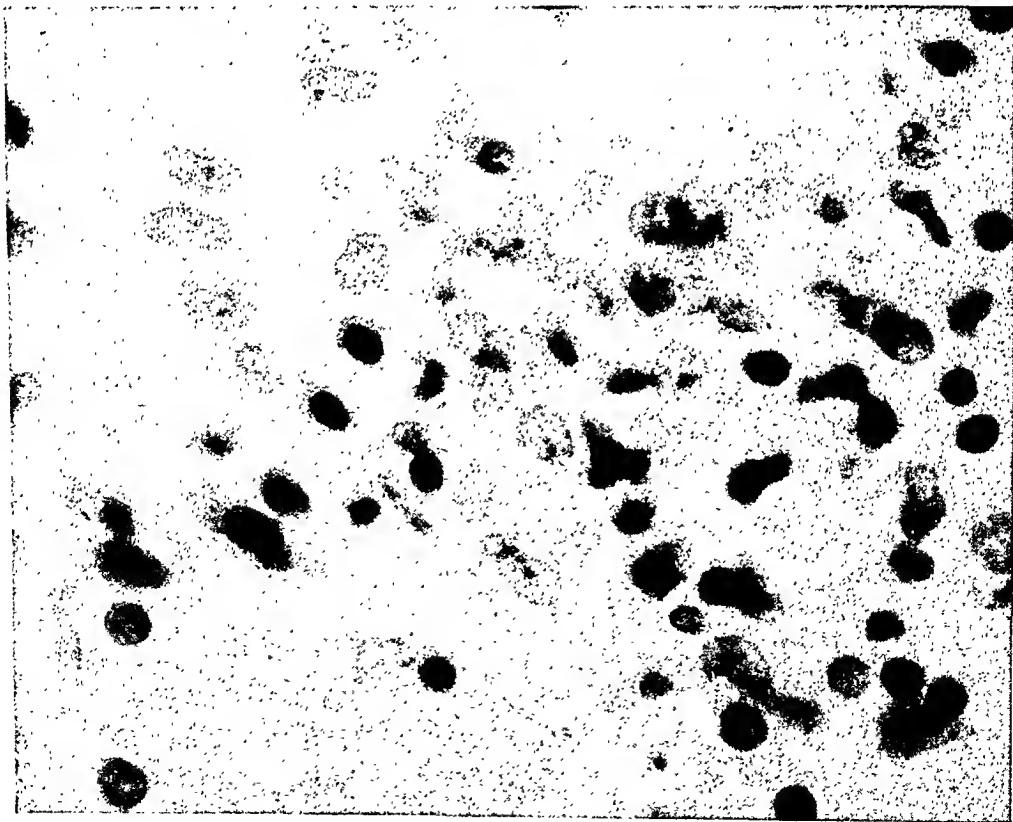
STUDIES ON CAPILLARY PERMEABILITY AS AFFECTED
BY ANOXEMIA *

Howard C. Hopps, M.D.,† and Julian H. Lewis, M.D.
(From the Department of Pathology, The University of Chicago, Chicago, Ill.)

The endothelial lining of blood vessels and lymphatics, especially that of capillaries, can be considered as a membrane which acts in great measure to preserve the integrity of the various organs and tissues, yet allowing for each to exert its proper effect upon the others and upon the organism as a whole. The mechanisms by which endothelial permeability is maintained normally or altered in certain pathologic states are thus of the utmost importance, both in health and disease.

Hypoxia ("anoxia") has long been recognized as a potent force for the production of increased permeability, and Landis [1] has beautifully demonstrated this effect and made quantitative determinations upon the extent of such action. He found that 3 minutes of oxygen lack increase filtration through the capillary wall fourfold and that not only did fluid escape in abnormal quantities but that considerable protein also was lost.

Much controversy has arisen over the part played by anoxemia in various types of edema, especially that due to cardiac failure. Current opinion is leaning more and more toward increased capillary pressure as the major factor in this condition, yet, as so aptly stated by Drinker and Yoffey,[2] "Even in well controlled experimental work the effects of venous obstruction, anoxemia, and carbon dioxide increase on capillary permeability cannot be separated from each other with finality; and in clinical conditions these three factors invariably operate together." Similarly, in considering the pathogenesis of surgical shock, the question invariably arises as to the importance of anoxemia and attendant increased capillary permeability. Only a few years ago, the mechanisms by which irreversible shock become established seemed relatively simple. It was thought that following an initial peripherovascular collapse, there developed a relative disproportion between circulating blood and the volume provided by the vascular bed. This resulted in decreased blood flow, stasis, anoxemia, and finally a marked increase in capillary permeability which allowed plasma to leak out into the tissues. A considerable number of experimental studies in the last

few years have shown conclusively that there is no generalized increased capillary permeability in shock.[3-7]

Maurer,[8] in a recent series of experiments, demonstrated that dogs which were subjected to low oxygen tensions responded by an increased production of cervical lymph and that there was an increased passage of protein from the blood capillaries to the lymph. This was accompanied by a corresponding decrease in the concentration of serum protein. McMichael and Morris [9] had previously demonstrated that "breathing mixtures containing percentages of oxygen as low as 9.5 is without effect on the rate of swelling of the human arm." For the most part, however, almost all of the experimental studies which have dealt with capillary permeability and anoxemia have been limited to observations on various localized regions in which a stagnation anoxemia was affected by obstruction to blood flow. Since capillaries of various tissues and regions of the body may behave quite differently, it is apparent that studies on capillary permeability in any local area may not necessarily indicate the reaction of capillaries elsewhere or of the capillary bed as a whole under similar conditions. Neither will the effects of stagnation anoxemia necessarily duplicate the effects of anoxic anoxemia.

With recent developments in the field of aeronautics, exposure to severe degrees of anoxia has become a more common occurrence. Experiments to determine the effects of general anoxemia of this type (anoxic anoxemia) on capillary permeability are therefore all the more important. We are concerned not only with the formation of edema and the pathogenesis of shock, but also with the question whether increased capillary permeability will permit the escape and localization in tissues of bacteria, toxins, antibody globulins, drugs, or other agents which may produce disease or modify inflammatory reaction. We are concerned, in fact, with any alteration of balance among those substances normally maintained in proper proportions by virtue of the semipermeability of capillary endothelium.

METHODS AND MATERIALS

In order to study capillary permeability, use was made of a phenomenon well known to immunologists, by means of which the escape of antibody globulin through blood capillaries may be determined. This phenomenon is illustrated as follows: If a guinea-pig is passively sensitized to some antigen, an appreciable interval of time (hours) must elapse before that animal becomes susceptible to anaphylactic shock, even though the sensitizing antibodies be administered directly into the blood stream. It is assumed that this so-called *minimum latent*

period of passive anaphylaxis is an expression of the time required for the antibodies to traverse the capillary endothelium and to permeate the tissues so that when the antigen-antibody reaction occurs, vital structures will be damaged and the changes characteristic of anaphylaxis will occur. If anoxic anoxia increases capillary permeability to antibody globulin, the minimum latent period of passive anaphylaxis should be correspondingly shortened.
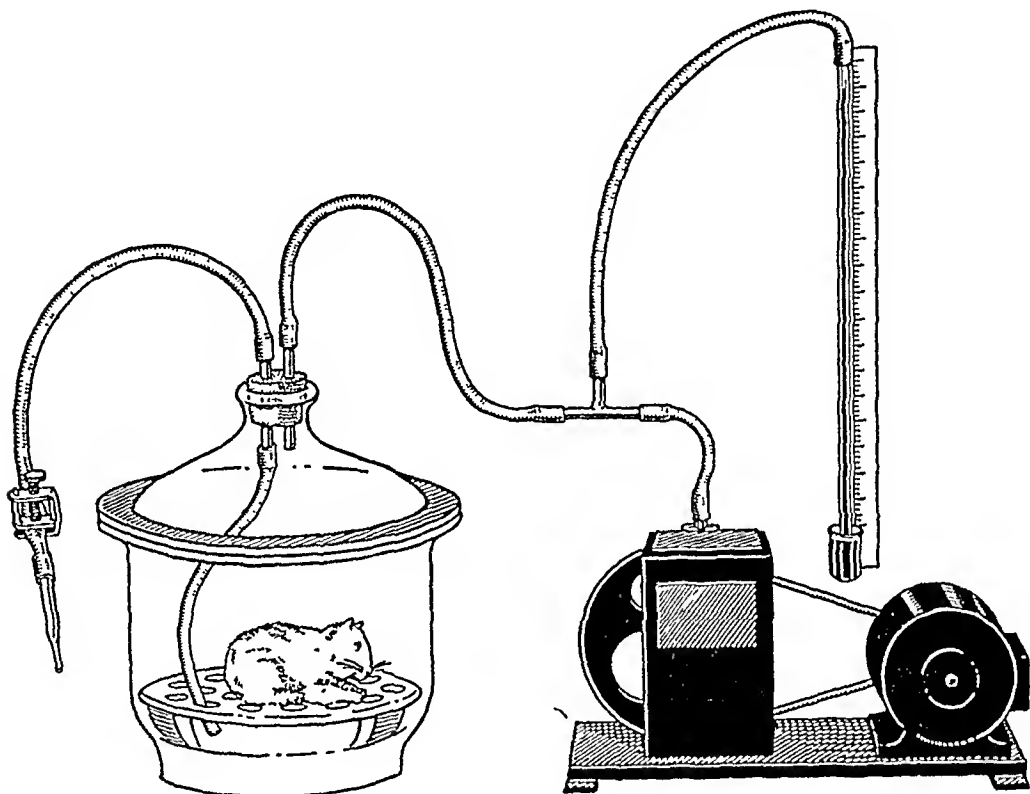
Text-Figure 1. Decompression chamber.

To produce anoxemia, a relatively simple mechanism was employed, one capable of duplicating rather closely such atmospheric conditions as would be encountered at high altitudes. It consisted of a large pyrex desiccator (8.5 liter capacity), the lid of which contained a two-hole stopper providing two large outlets. One of these outlets was connected with a vacuum pump; the other admitted a rubber tube, one end of which led to the bottom of the chamber, the other end projected to the outside and was fitted with a screw clamp in order that an adequate intake of air could be allowed, and yet the pressure within the chamber could be reduced to, and maintained at, any desired level. A mercury manometer was introduced into the system (Text-Fig. 1). Preliminary studies showed that an atmospheric pressure of 230 mm. Hg, equivalent to 30,000 feet above sea level, was near the limit of toler-

ance for guinea-pigs when maintained at this pressure for a period of 30 minutes. This pressure and exposure time were used throughout all of the experiments. At this pressure, O_2 concentration is 6.4 per cent.[10] The chamber was evacuated at a steady and closely regulated rate during a period of 15 minutes, at which time the desired oxygen concentration ("altitude") was attained.

Young, healthy, adult guinea-pigs were used throughout the experiments, and three were placed in the chamber at one time. In anaphylactic studies, a single source of high titer pooled anti-crystalline-egg-albumin rabbit serum was used. The sensitizing dose of antiserum and the shocking dose of 1 per cent crystallized egg albumin were administered via the jugular vein.

In experiments in which determinations on blood plasma were required, either heparin or a mixture of ammonium and potassium oxalate was used as the anticoagulant. Protein determinations were done by the densimeter method of Barbour and Hamilton.[11] Hemoglobin determinations were made using the Dick-Stevens photo-electric hemoglobinometer.

EXPERIMENTAL OBSERVATIONS

Preliminary experiments showed that, with the particular antiserum used (anti-crystalline-egg-albumin rabbit serum), 0.01 cc., given intravenously, was the minimum lethal sensitizing dose when a shocking dose of 1 cc. of 1 per cent crystallized egg albumin was given 24 hours later; 0.001 cc. of this antiserum would result in a definite but non-fatal anaphylaxis under these conditions. The minimum latent period for fatal anaphylaxis when 0.02 cc. of antiserum was given intravenously was 126 to 135 minutes, although with larger sensitizing doses this minimum latent period could be considerably shortened. In the anaphylactic studies to be described, 0.02 cc. of antiserum (2 times the minimum sensitizing dose) was routinely employed as the sensitizing dose; 1.0 cc. of 1 per cent crystalline egg albumin was the shocking dose. Sensitized control animals were left at normal atmospheric pressure. Treated animals, 5 minutes after sensitization, were placed in the pressure chamber and exposed to an atmospheric pressure equivalent to that found at 30,000 feet ($O_2 = 6.4$ per cent). Table I illustrates the results of such experiments on 20 animals. It is evident that the minimum latent period was not shortened as a result of anoxemia in the 9 treated animals.

Since antibody globulin is one of the larger protein molecules of the plasma, it was considered that some smaller protein molecule, such as serum albumin, might leak through capillaries under these conditions of anoxemia even though globulin did not appear to do so. Conse-

quently, investigations were undertaken utilizing the dye T-1824 (Evans blue). It has been demonstrated [12] that this dye, when mixed with blood or serum, behaves like serum albumin * in so far as its permeability to various membranes is concerned. Five series of experiments on carefully paired (control and treated) guinea-pigs were performed. Paired animals were of almost identical weight and, within each pair, the interval between injection of the dye and the collection of blood samples was the same, ±30 seconds. In order to minimize trauma

TABLE I

Minimum Latent Period for Fatal Anaphylactic Shock in Passively Sensitized Guinea-Pigs

Controls	Interval	Anoxic
	(minutes)	
	90	0
	108	00
	118	0+
0	120	
++	126	0
	128	+
00	132	
	133	0
++	135	
	136	+
++	137	
+	171	
+	180	

+ indicates fatal anaphylaxis; 0 indicates survival. (Each symbol represents one animal.)

(cardiac puncture) and to protect the animals from preliminary blood loss, which would induce a shift in interstitial fluids and rather marked hemodilution, reliance was placed upon the law of averages to provide relatively equal blood-dye concentration in the control and treated groups following the introduction of a constant dose of T-1824 per kg. of body weight. Determinations of the dye T-1824 were done on plasma. A sufficient amount of dye was injected so that the sample of plasma obtained could be diluted 1:10, in order to minimize blood loss. The dye containing plasma was diluted with beef plasma, and determinations were done with the Evelyn and with the Klett Summerson photo-nephelometers. Samples were centrifuged immediately before reading. All animals received 4 mg. per kg. of body weight of T-1824 intravenously (jugular vein). This material was carefully prepared in a volumetric flask so that 1 cc. of the saline solution contained 1 mg. of the dye. Injections were made with a syringe of tuberculin type, graduated in 0.01 cc.

* With the concentrations of T-1824 used in these experiments, all of the dye would not have been bound to the albumin fraction although the major part of the dye would have been incorporated in the serum albumin.

In one experiment (Table II) only hemoglobin and protein determinations were made. These were made both before and after subjecting the animals to reduced O_2 tension and required less than 1 cc. of blood from each animal. Their purpose was to evaluate any hemoconcentration or dilution brought about by this acute anoxemia which might in turn affect the blood-dye concentration of anoxic animals (see Table II).

TABLE II

The Effect of Anoxic Anoxemia on Disappearance Rate of T-1824, Hemoglobin, and Plasma Proteins

Series	Animals	Average concentration		
		T-1824	Hemo-globin	Protein
		(mg. %)	*(gm. %)*	*(gm. %)*
Series 1, paired guinea-pigs: 380–486 gm.; interval, 77'–88'*	Anoxic (3) Controls (3)	7.525 7.465		
Series 2, paired guinea-pigs: 363–446 gm.; interval, 87'–88'*	Anoxic (3) Controls (3)	6.62 5.67		
Series 3, guinea-pigs: 380–454 gm.; interval, 77'–87'†	Before anoxia (6) After anoxia (6)		14.0 12.4	5.39 4.51‖
Series 4, paired guinea-pigs: 336–467 gm.; 137'–161'*	Before anoxia (6)‡ After anoxia (6)	4.64		5.29 4.54
	Controls: 1st dtmn. (6)§ Controls: 2nd dtmn. (6)§	4.64		5.30 4.70

* Interval of time in minutes elapsing between injection of T-1824 and withdrawal of blood sample.

† Interval of time in minutes elapsing between onset of anoxemia and withdrawal of second blood sample.

‡ Blood sample withdrawn 20 minutes after injection of T-1824.

§ First and second determinations parallel, in time, the withdrawal of blood samples "before" and "after" anoxia.

‖ It may be considered that the variation in hemoglobin in series 3 is a measure of hemodilution resulting from treatment. If protein determination "after anoxia" is corrected on this basis, the decrease in plasma protein is actually 6.1%.

In a fifth experiment, dye, hematocrit, and protein determinations were made in both control and treated animals before and after one-half of the animals were subjected to reduced O_2 tension. Any differences in ratios of blood volume to body weight, the result of hypoxia, could thus be determined and dye and protein concentrations could be corrected for these differences (see Table III).

From these observations it appears that the rate of disappearance of T-1824 is not increased in animals which are subjected to acute anoxic anoxia. There is a suggestion that the rate actually may be decreased as a result of anoxemia. In the one series in which plasma protein determinations were made before and after animals were exposed to decreased O_2 tension and compared with similar determinations in control animals, *no significant variation was observed (Table III).*

DISCUSSION

On the basis of evidence presented here, one may conclude that acute anoxic anoxia, of a degree which approximates or slightly exceeds the minimal lethal range for human beings, does not produce a detectable increase in capillary permeability to plasma proteins in guinea-pigs, although three entirely different methods were employed to determine such an effect. These findings correlate well with similar observations

TABLE III

The Effect of Anoxic Anoxemia on Disappearance Rate of T-1824, Plasma Protein, and Hematocrit Determination

Series	Animals	Average concentration		
		T-1824	Protein	Hemato-crit
Series 5, paired guinea-pigs: 575–740 gm.; interval,* 112′–114′	Before anoxia (5)† After anoxia (5)	(mg. %) 9.39 6.10	(gm. %) 4.55 3.81	(%) 41.1 36.1
	Controls: 1st dtmn. (5)‡ Controls: 2nd dtmn. (5)‡	10.03 6.47	4.85 4.31	42.5 38.1

* Interval of time elapsing between injection of T-1824 and withdrawal of second blood sample.

† Blood sample withdrawn 20 minutes after injection of T-1824.

‡ First and second determinations parallel, in time, the withdrawal of blood samples "before" and "after" anoxemia. It may be considered that the variation in hematocrit reading is a measure of hemodilution resulting from treatment. When the second determinations of T-1824 and of protein are corrected on this basis, the variation is:

Animals	T-1824	Protein
Anoxic (5)	−26.1%	−4 6%
Controls (5)	−28.1%	−0.8%

on the state of capillary permeability in conditions of surgical shock.[3-7] In the careful experiments of Landis,[1] and others, which have shown an increased regional capillary permeability from stagnation anoxemia, it seems probable that alteration in carbon dioxide was the major factor in producing this effect.

SUMMARY AND CONCLUSION

1. The minimum latent period for anaphylactic shock in guinea-pigs following passive sensitization is presumed to be an indication of the time necessary for antibodies to escape from the blood stream into the tissues. This minimum latent period was not shortened by acute anoxic anoxemia brought about by subjecting passively sensitized guinea-pigs to low oxygen tensions. Therefore, anoxemia, under these conditions, does not facilitate the passage of antibody globulin through vascular endothelium.

2. Studies on the rate of disappearance of T-1824 from the blood stream indicate that acute anoxia does not increase the normal rate of disappearance of this dye. This dye, when mixed with blood or serum, behaves like serum albumin in so far as its permeability to various membranes is concerned. Therefore, anoxemia, under the conditions described, does not facilitate the passage of serum albumin through vascular endothelium.

3. Significant alterations in the quantity of plasma protein following acute anoxic anoxia were not observed.

REFERENCES

1. Landis, E. M. Micro-injection studies of capillary permeability. III. The effect of lack of oxygen on the permeability of the capillary wall to fluid and to the plasma proteins. *Am. J. Physiol.*, 1927–28, 83, 528–542.
2. Drinker, C. K., and Yoffey, J. M. Lymphatics, Lymph, and Lymphoid Tissue. Harvard University Press, Cambridge, 1941, p. 311.
3. Engel, D., and Forrai, E. Capillary permeability in traumatic shock. *J. Physiol.*, 1943–44, 102, 127–139.
4. Fine, J., and Seligman, A. M. Traumatic shock. IV. A study of the problem of the "lost plasma" in hemorrhagic shock by the use of radioactive plasma protein. *J. Clin. Investigation*, 1943, 22, 285–303.
5. Fine, J., and Seligman, A. M. Traumatic shock. VII. A study of the problem of the "lost plasma" in hemorrhagic, tourniquet, and burn shock by the use of radioactive iodo-plasma protein. *J. Clin. Investigation*, 1944, 23, 720–730.
6. Evans, E. I., Hoover, M. J., James, G. W., III, and Alm, T. Studies on traumatic shock. I. Blood volume changes in traumatic shock. *Ann. Surg.*, 1944, 119, 64–85.
7. Ashworth, C. T., Jester, A. W., and Guy, E. L. Local loss of fluid and protein in experimental shock: relation to decrease of plasma volume and total circulating protein. *Am. J. Physiol.*, 1944, 141, 571–574.
8. Maurer, F. W. The effects of decreased blood oxygen and increased blood carbon dioxide on the flow and composition of cervical and cardiac lymph. *Am. J. Physiol.*, 1940, 131, 331–348.
9. McMichael, J., and Morris, K. M. Acute oxygen lack and capillary permeability in man. *J. Physiol.*, 1936, 87, 74-P.
10. Van Liere, E. J. Anoxia, Its Effect on the Body. The University of Chicago Press, 1942, p. 17.
11. Barbour, H. G., and Hamilton, W. F. The falling drop method for determining specific gravity. *J. Biol. Chem.*, 1926, 69, 625–640.
12. Rawson, R. A. The binding of T-1824 and structurally related diazo dyes by the plasma proteins. *Am. J. Physiol.*, 1943, 138, 708–717.

THE PATHOLOGY OF MALIGNANT CATARRHAL FEVER
(BOVINE EPITHELIOSIS)

With Special Reference to Cytoplasmic Inclusions *

Leonard W. Goss, D.V.M., Clarence R. Cole, D.V.M., and Robert E. Kissling, D.V.M.

(From the Department of Pathology, College of Veterinary Medicine, Ohio State University, Columbus, Ohio)

Malignant catarrhal fever is an infectious, noncontagious, sporadic, highly fatal disease of cattle. Since the successful transmission of the disease by blood inoculation reported by Goetze and Liess,[1] the etiologic agent has been thought to be a filterable virus. However, their filtration experiments and inoculations with blood plasma gave only negative results. Others were able to produce the disease in cattle by intravenous and subcutaneous inoculation of whole blood, but filtrates of such blood were not infective. No microorganisms could be demonstrated in the whole blood used for inoculation.[2]

In their studies in South Africa, du Toit and Alexander[3] reported nonfilterability of the virus and explained this characteristic by suggesting that the virus was closely associated with erythrocytes.

Since cell inclusions are found in many virus diseases, a detailed search was made for their presence in malignant catarrhal fever. Of 18 cases of this disease occurring in one community, a detailed study of the pathologic changes was made in 3. This search revealed inclusion bodies in numerous tissues in all cases. The finding of these cytoplasmic cell inclusions in these cases should help establish the viral causation. Demonstration of inclusions may become a laboratory procedure which will aid in a positive diagnosis of this disease.

Clinical Data

The history and symptoms of all cases were similar. Animals of both sexes and ages from 10-months-old calves to mature animals were affected. The course varied from 5 to 14 days, and only 2 of 18 affected animals recovered. All cases occurred on different farms except for 4 which were on two farms on each of which 2 animals were affected.

Excessive lacrimation, congestion of the sclera and conjunctiva, extreme dejection, dysphagia, dyspnea, and a temperature of 106° to 108°F. were noted on the first day. Later, cloudiness of the cornea occurred, as well as excessive salivation, with papule and vesicle formation on the muzzle, skin, and buccal, labial, gingival, and pharyngeal mucosa. The serous inflammation of the oral, nasal, and vaginal mu-

cosa changed to sero-catarrhal, then catarrhal or croupous, and finally there were patches of epithelial excoriation from the muzzle and from the labial, pharyngeal, and gingival regions. A fetid odor was emitted from the mouth and nostrils due to necrosis of the muzzle and mucous membranes.

The vesicles in the skin ruptured, leaving moist areas, followed by drying of the serous fluid and epithelium, forming parchment-like crusts in the hair. Near the termination of the disease, the corium of the horns became detached from the corneal processes when bumped or grasped.

Gross Findings

The anatomic diagnoses included: papular and vesicular dermatitis, especially pronounced on the withers, forearms, scrotum, and teats; easy detachment of the corium of the horns from the cornual processes; serous, sero-hemorrhagic, croupous and diphtheritic stomatitis, pharyngitis, rhinitis, and vaginitis; swollen submaxillary and anterior cervical lymph glands; and conjunctivitis. The corneas were opaque and more than twice as thick as is normal.

Histopathologic Findings

Tissues were fixed in 10 per cent formalin, embedded in paraffin, cut at 4 μ, and stained with hematoxylin and eosin or Shorr's III.[4] The tissues were taken from animals *in extremis*.

The nasal mucosa showed extensive desquamation of the epithelium with congestion and hemorrhage in the submucosa. Cytoplasmic, homogenous, acidophilic, and basophilic inclusions were observed in the epithelial cells.

The pharyngeal mucosa and tongue showed numerous vesicles and submucosal hemorrhage. Karyorrhexis and pyknosis of the nuclei of the epithelium were present. In all tissues there was practically no leukocytic response to the damage incurred, only a mild eosinophilic leukocytic infiltration of the submucosa being observed.

The opacity of the cornea noted grossly was due to edema in the substantia propria. The connective tissue lamellae of the substantia propria were separated by wide spaces filled with edema fluid, resulting in a cornea measuring from 2.5 mm. (center) to 4.6 mm. (periphery) in thickness. Normal corneas taken from animals after slaughter measured 1.0 mm. to 1.8 mm. in thickness.

Sections of the brain showed petechial hemorrhages of the cerebrum and cerebellum. The Purkinje cells were degenerated and an occasional acidophilic cytoplasmic inclusion was observed in these cells. Perivascular glial accumulations were seen in the white matter of the cerebellum.

Blood smears showed marked erythrocytic anisocytosis.

Smear preparations were made of scrapings from the conjunctival, nasal, oral, and pharyngeal mucosa to determine the presence of inclusion bodies. When stained with Shorr's III [4] or hematoxylin and eosin, three general types of inclusions were observed in the cytoplasm of the epithelial cells. Most numerous were cells showing diffuse, granular, basophilic, cytoplasmic inclusions which measured 0.25 to 0.50 μ in diameter. The number of inclusions per cell varied from only a few, to some instances in which the cytoplasm was packed with these bodies. The second type of inclusion observed consisted of clustered, granular, basophilic cytoplasmic bodies measuring from 1.75 to 5.25 μ in diameter. The granules composing these bodies were of the same size as those observed scattered diffusely throughout the cells described above. The number per cell varied from one to fifteen. Often the cells possessing the clustered inclusions also contained several of the scattered granules. The third type of inclusion consisted of sharply defined homogeneous bodies usually staining acidophilic, although some were neutrophilic. These measured from 2.0 to 5.25 μ in diameter, the majority being 3.5 μ. According to the work of Lucas and Riser [5] on inclusions of panleukopenia (infectious enteritis) of cats, these types may represent various stages in the formation of the mature homogeneous inclusion, starting with diffuse granular bodies.

Shorr's III stain [4] gave the most satisfactory results, offering a sharper distinction to the inclusion. Hematoxylin and eosin stain also gave fairly satisfactory results. Giemsa's stain gave poor differentiation between cytoplasm and inclusions. Seller's [6] stain failed to stain the inclusions. Azur II and eosin failed to give cellular detail.

For critical study of cellular detail, a binocular Zeiss microscope was employed using the apochromatic 120 $\times$, 1.30 n.a. objective and 10 $\times$ compensating eyepieces. Additional magnification of 1.5 $\times$ was given by the Zeiss binocular. The 1.2 condenser was always focused as carefully as the objective. A Bausch and Lomb gas-filled projection mazda lamp was the source of illumination.

SUMMARY AND CONCLUSIONS

Malignant catarrhal fever is characterized primarily by a serous or sero-catarrhal inflammation largely involving the epithelial tissues, followed by varying degrees of catarrhal, croupous, and diphtheritic inflammation of the mucous membranes. It is accompanied by neuronal degeneration and hemorrhage in the brain.

Cytoplasmic inclusion bodies are present in the epithelial cells of mucous membranes. Three types were observed: diffuse granular, clustered granular, and homogeneous.

The finding of cytoplasmic inclusions in the epithelial cells adds to the evidence supporting viral causation.

The demonstration of these cytoplasmic inclusions may be a laboratory procedure which will aid in differentiating malignant catarrhal fever from vesicular stomatitis, rinderpest, acute infectious aphtha, and aphthous stomatitis.

Since the lesions involve epithelial structures and the inflammation is primarily of a serous or sero-catarrhal type, it is suggested that bovine epitheliosis is a more appropriate and descriptive name than malignant catarrhal fever.

REFERENCES

1. Goetze, R., and Liess, J. Erfolgreiche Uebertragungsversuche des bösartigen Katarrhalfiebers von Rind zu Rind. Identität mit der Südafrikanischen Snotsiekte. *Deutsche tierärztl. Wchnschr.*, 1929, 37, 433–437.

2. Editorial. Le coryza-gangréneux á la Martinique. *Rec. Med. Vét. exot.*, 1937, 10, 155–158. (Abstract in: *Vet. Bull.*, 1938, 8, 506–507.)

3. Du Toit, P., and Alexander, R. A. Malignant Catarrhal Fever and Similar Diseases. 13th International Veterinary Congress 1938, Section III, pp. 15–21.

4. Page, W. G., and Green, R. G. An improved diagnostic stain for distemper inclusions. *Cornell Vet.*, 1942, 32, 265–268.

5. Lucas, A. M., and Riser, W. H. Intranuclear inclusions in panleukopenia of cats. A correlation with the pathogenesis of the disease and comparison with inclusions of herpes, B-virus, yellow fever, and burns. *Am. J. Path.*, 1945, 21, 435–465.

6. Simmons, J. S., and Gentzkow, C. J. Laboratory Methods of the United States Army. Lea & Febiger, Philadelphia, 1944, ed. 5, p. 754.

DESCRIPTION OF PLATE

PLATE 132

FIG. 1. Epithelium, nasal mucosa. Neutrophilic homogeneous cytoplasmic inclusions. Hematoxylin and eosin stain. × 1800.

FIG. 2. Epithelium, conjunctival mucosa. Acidophilic homogeneous cytoplasmic inclusions. Hematoxylin and eosin stain. × 1800.

FIG. 3. Epithelium, pharyngeal mucosa. Clustered granular and diffuse granular cytoplasmic inclusions. Shorr's III stain.[4] × 1800.

FIG. 4. Epithelium, oral mucosa. Homogeneous and granular inclusions. Shorr's III stain.[4]

FIG. 5. Pharyngeal mucosa. Vesicle. Hematoxylin and eosin stain. × 150.

FIG. 6. Nasal mucosa. Catarrhal rhinitis. Hematoxylin and eosin stain. × 150.

PLATE 132

Bovine Epitheliosis
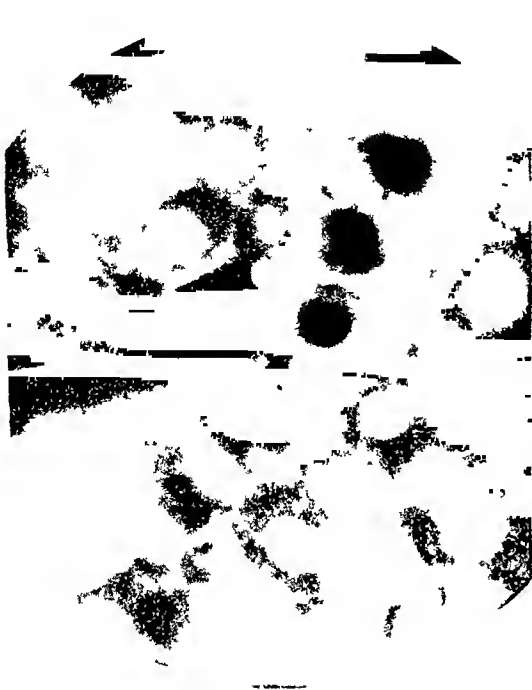
DEVELOPMENT OF THE INFLAMMATORY LESIONS AND OF RICKETTSIAE OF MURINE TYPHUS IN THE LUNGS OF RATS *

WALENTY NYKA, M.D.

(*From the Emergency Vaccine Laboratory, East Everleigh, Wilts., England, and the Laboratorio del Tifo, Hospital General, Mexico, D.F.*)

Although, during recent years, important progress has been made in our knowledge of the typhus virus, the mode of multiplication of rickettsiae remains obscure. Various theories have been proposed. One of the most common is the ultravirus theory advocated by Chandler [1] and others, which assumes that the smallest visible forms of the pleomorphic typhus virus are in continuity with others, smaller still, invisible with the ordinary microscope. It finds support in the difficulty or even impossibility of finding any rickettsiae in the earliest typhus lesions developing in certain animals. On the other hand, Begg, Fulton, and van den Ende [2] suggested the existence of a developmental cycle, somewhat similar to that of psittacosis virus.[3] In this cycle, especially during the period of adaptation to a new host, the virus passes from the earliest stage of "homogeneous inclusion bodies" to the stage of "morulae," which mature progressively to reach the adult stage when the limiting membrane is disrupted and the rickettsial masses are discharged. To elucidate the way in which rickettsiae multiply, a series of experiments has been carried out, with the results here recorded.

METHOD

Rats were infected according to Castañeda's method.[4] One cc. of a suspension, prepared by grinding the lung of a mouse infected with murine typhus in 4 cc. of 10 per cent horse serum broth and storing at —76°C. in a mixture of alcohol and solid carbon dioxide, was introduced intranasally. One animal died 3 minutes after infection, the others were killed with chloroform after 1, 3, 5, 7, 9, 24, 33, 48, and 55 hours; between the 72nd and the 96th hours most of the animals died, the survivors being killed with chloroform. The rickettsiae were stained with methyl violet and metanil yellow [5]; the sections for examination of the inflammatory lesions, with hematoxylin and eosin.

DEVELOPMENT OF LESIONS IN THE LUNG

Macroscopic Appearance. Three minutes after infection, the lungs were congested, particularly the upper and central parts around the hilum. As time passed, the congestion became more marked and progressively involved the entire lung, being followed, after 48 hours, by

---

foci of consolidation which increased in size and became confluent, so that in animals dying between the third and fourth days large areas of grayish red to deep red consolidation were found. Moreover, exudate, which is a marked feature in mice, was never really abundant except sometimes in the pyramidal lobe at the terminal stage of the inflammatory process. In the pleural cavity exudate has never been observed.

Microscopic Appearance. Three minutes after infection, the capillaries of the lung were dilated and numerous alveoli were filled with the inoculum. In lungs fixed 1 hour later, polymorphonuclear leukocytes were present in increased numbers in the capillaries and a few were seen in the cellular interstices of the alveolar septa. Some of the alveolar cells were slightly swollen and a few were desquamated into the alveolar spaces. After 3 hours, the swelling of the cells increased, more cells were involved, and many of the alveolar spaces were narrowed. Exudation of fluid into the alveoli began at this early stage. Five hours after infection, groups of alveoli lined with enlarged alveolar cells and surrounded by almost normal alveoli were seen. In these foci the alveolar spaces contained desquamated alveolar cells in greater numbers and also a few polymorphonuclear leukocytes. After 7 hours, cuffs of small lymphocytes mixed with a few polymorphonuclear leukocytes appeared around the blood vessels of the bronchioles. After 9 hours, the alveolar spaces in the focal areas were filled with swollen and desquamated alveolar cells, and inflammatory edema was abundant. Twenty-four hours after infection, plasmacytes appeared around the capillaries; the foci had increased in size and contained a greater number of obliterated alveoli. After 33 hours, and still more after 48 hours, the foci of consolidation merged into one another, more parenchyma being thus progressively involved in the inflammatory process, which reached its full extent and intensity 72 hours after infection. At this stage, large portions of the lung were consolidated, the alveolar spaces being filled with swollen desquamated alveolar cells, leukocytes, and inflammatory edema. The remaining portions of lung were congested and might contain a few foci of consolidation. All over the lung, but particularly in the consolidated portions, numerous polymorphonuclear leukocytes, lymphocytes, and plasmacytes were seen, singly or in groups. Thick cuffs of small monocytes surrounded the bronchioles and blood vessels, together with variable numbers of the aforementioned cells. The bronchial epithelium showed some swelling, particularly at the terminal stages, but a massive desquamation never occurred. The bronchioles were filled with leukocytes and desquamated alveolar cells, among which a few bronchial epithelial cells and cellular

débris were found. The pleura over nonconsolidated areas showed no change, but over consolidated parts the mesothelial cells were enlarged and often vacuolated; sometimes they degenerated and disappeared.

Development of the Rickettsiae

Three minutes after infection, rickettsiae were found in the inoculum filling the alveoli and scattered among the cells of the interalveolar septa (Fig. 1). In all animals examined, they were more numerous in the upper and central than in the lower portions of the lungs. Dot forms and coccobacillary types predominated, diplococci and rod forms were scanty. At that stage the organisms stained with great difficulty. After 1 hour, fairly numerous rickettsiae could be seen inside the alveolar cells or on their surface (Fig. 2). As time passed they appeared in increasing numbers in the alveolar cells and in the epithelial cells of the bronchial tree, becoming gradually less numerous, though never absent from the alveolar spaces. At this early stage the rickettsiae began to undergo important morphologic changes, dots becoming less numerous while coccobacilli and rods increased in number. The morphologic changes were visible after 3 hours (Fig. 3), but they became conspicuous after 5 hours. At this stage small, irregularly shaped, dense clumps appeared in which the organisms, unlike those composing the loose groups probably of purely mechanical origin and which were seen 3 hours after infection (Fig. 3), could not be seen along their whole length (Fig. 4). These clumps were situated in the interalveolar septa, inside the alveolar cells or on their surfaces. At this stage, diplobacillary forms of rickettsiae appeared, twice as large as a coccobacillus (Fig. 4 insert), and also rod forms which were considered to be of the adult type since their length and staining properties were similar to those which are commonly found at the terminal stage. Seven and 9 hours after infection, the adult rods were more numerous and the clumps increased both in number and size (Figs. 5 and 6). Some of the clumps, fairly numerous but still irregular in shape after 24 hours (Fig. 7), acquired, after 33 hours, a rounded shape and a granular structure and formed small granular bodies, while those remaining retained their irregular shape (Fig. 8). At 48 hours, the differentiation of the clumps into two types of unlike structure, the granular bodies and the irregularly shaped aggregates, became conspicuous (Fig. 9). The granular bodies were characterized by a rounded form, sharp edges, and dense structure; the irregularly shaped aggregates were ill defined and usually of less dense structure than the granular bodies. From both, individual rickettsiae grew out into neighboring cellular interstices and alveolar spaces. Fifty-five hours after infection, granular bodies and

aggregates had increased considerably in size (Fig. 10), and after 72 hours they had reached their full size (Fig. 11). During this period, single rickettsiae, grown out in large numbers from the infected cells, infiltrated tissue interstices and alveoli filled with inflammatory edema and numerous organisms. There was, however, considerable variation in the number of rickettsiae in individual animals.

Rickettsiae do not necessarily develop in clumps. From the early stages many alveolar cells contained individual rickettsiae which multiplied actively, so that their cytoplasm was first dotted with individual organisms and later filled with diffuse masses of rickettsiae from which individual organisms grew out into the neighboring alveolar spaces (Fig. 32). This diffuse development could be observed only in certain alveolar cells, but it was common in the bronchial epithelial cells. It was difficult to decide when the multiplication of rickettsiae began in these cells, since in the early stages they might increase in number within the cells either by multiplication or by the inclusion of new organisms.

In animals killed 72 to 96 hours after infection, extracellular and intracellular rickettsiae were very numerous, being more abundant in the consolidated (Fig. 12) than in the nonconsolidated (Fig. 13) portions of the lungs. Extracellular rickettsiae could be seen particularly in the tissue interstices and in the inflammatory edema filling the alveoli, either singly (Fig. 14) or in clumps (Fig. 15). Stained purple, the organisms stood out sharply from the yellow-stained exudate, and all forms mentioned above could readily be made out, with numerical predominance of the longer forms over the dots (Fig. 14). Quite often, single rickettsiae and clumps coexisted within the same alveolar space, with no sharp limits between them (Fig. 15). In some alveoli which had become confluent through rupture of the interalveolar walls, very large and fairly well defined rickettsial masses were seen which were about twenty times the size of an alveolar cell. These masses were similar to those which have been described in mice, but most of them were more voluminous.[5] Quite often, rickettsiae were seen at the peripheries of these masses, singly or in clumps, scattered in the surrounding inflammatory edema. The intracellular rickettsiae, as in mice, were particularly numerous in the alveolar cells. Almost all of these cells were infected in the terminal stages, but the number and arrangement of the organisms within them varied greatly. Some cells contained only a few organisms (Figs. 16 and 17); some were partly (Figs. 18, 19, 20, 23, and 26) and others entirely (Figs. 21, 22, and 27) filled with diffuse rickettsial masses. Besides these cells, others could be seen which contained in their cytoplasm one (Fig. 17) or several (Fig. 18) aggregates. If a cell contained several aggregates, they might be arranged at the

periphery (Fig. 18), or around the nucleus which might be surrounded by rickettsiae. Quite often, aggregates could be seen on the surface of the cytoplasm of the harboring cells, protruding into the cellular interstices (Figs. 24 and 25). In some cells aggregates coexisted with single rickettsiae (Fig. 17). Numerous cells contained granular structures similar to those described in mice.[5] In rats, too, the granular bodies varied greatly in size, shape, and structure. Besides granular bodies not larger than the smallest blood-platelet, others could be seen as large as those shown in Figures 28 and 29, distending and entirely filling the cytoplasm of the harboring cells. Usually round or oval, they might be fusiform, pyriform, triangular, beaded, or otherwise irregular. Often they had one or more rounded, lateral projections (Fig. 29). Although most of them were dense in structure, in some granular bodies the organisms were arranged less closely.

No strict separation could be made among the irregularly shaped aggregates, the diffuse rickettsial masses filling the cells, and the granular structures; intermediate forms were readily found (Fig. 27). Frequently, aggregates and granular bodies coexisted in the same cells (Fig. 30). Sometimes the granular bodies were studded with clear spots exhibiting the staining affinities of cytoplasm (Fig. 29). In no case was any limiting membrane seen. Most of the bronchial epithelial cells were stuffed with diffuse masses of rickettsiae. It seems worth noting that these cells never developed characteristic granular bodies, and that no cells containing only a few rickettsiae could be made out at the terminal stages; either they were packed with organisms or free of them. In and among the desquamated cells and cellular débris filling the bronchioles, innumerable rickettsiae could be seen, sometimes in clumps but usually single and normal in appearance. Such granular bodies as could be seen in certain desquamated alveolar cells showed no sign of alteration in their structure. In rats, as in mice, the polymorphonuclear leukocytes contained comparatively few rickettsiae, usually coccobacilli and rods, normal in appearance. Endothelial cells of the capillaries rarely showed rickettsiae. If they were infected, their cytoplasm was filled with uniform masses of rickettsiae or with small but numerous granular bodies. The infected cells were swollen and projected into the lumina of the capillaries.

DISCUSSION

Nature of the Inflammatory Process

This study shows the primarily focal character of rickettsial bronchopneumonia in rats, which differs from that found in mice only in its lesser intensity and extent, while the inflammatory edema also is less abundant. The cellular changes, however, show a noticeable difference.

The swelling of the alveolar cells is always very marked and desquamation of the swollen cells resulting in obliteration of numerous alveolar spaces is a prominent feature. The abundant desquamation and elimination of infected alveolar cells which, mixed with numerous polymorphonuclear leukocytes containing phagocytized rickettsiae and a great number of single rickettsiae, are found filling most of the bronchioles, can be regarded as a strong cellular reaction of the rat against rickettsial infection. This idea is strengthened by the fact that polymorphonuclear leukocytes appear as early as 1 hour after infection. Although no bacteriologic examinations have been carried out to eliminate the possibility of coexistent bacterial infection, the reaction seems to be directed against the rickettsiae since no bacteria have been found in the sections.

Development of Rickettsiae

Four phases can be distinguished in the development of rickettsiae in the lungs of rats: (1) they appear within the cells, (2) they undergo morphologic changes, (3) they multiply, (4) they grow out from the infected cells. Whatever may be the mechanism by which rickettsiae become intracellular, it is certain that very soon after the introduction of the rickettsial suspension into the lung many alveolar cells become infected, and that the number of intracellular organisms increases progressively, while extracellular organisms become less numerous. Parallel with this, extracellular as well as intracellular rickettsiae undergo morphologic changes, the dot forms becoming less numerous, while the coccobacilli and rods increase in number. The most striking morphologic change is the appearance, 5 hours after infection, of adult rods and diplobacilli. Their appearance is associated with that of small intracellular clumps which are considered to be the earliest colonies of rickettsiae. It is therefore difficult to escape the conclusion that morphologic changes of the rickettsiae are related to their multiplication. The colonies are thus assumed to originate from the multiplication by transverse fission of single rods or diplobacilli, the new organisms remaining together, multiplying in their turn, and thus increasing the size of the colonies. This view is supported by the fact that rods and diplobacilli very often are found in the neighborhood of early colonies (Fig. 5). It is impossible to say whether the dot forms have to go through the stages of coccobacilli, adult rods, and diplobacilli before multiplying, or whether they can multiply directly. When the clumps have reached a certain size, they differentiate into granular bodies and irregularly shaped aggregates; in later stages, individual rickettsiae grow out of them in increasing numbers until, in the terminal stages, all cellular interstices are invaded.

No stage has been encountered in which rickettsiae are absent. Therefore, it appears unnecessary to postulate the existence of an ultramicroscopic phase.

Extracellular Growth of Rickettsiae

Once set free, rickettsiae may gain entrance into other cells and grow within them, or remain extracellular and multiply. The extracellular growth is demonstrated by the development of numerous extracellular clumps of organisms such as are shown in Figure 15. These, clumps are considered to originate from the multiplication of single extracellular rickettsiae and, therefore, to be colonies of rickettsiae similar to those described inside cells. In support of that view it can be put forward that the size of these clumps is not fixed but increases progressively. In Figure 15, the whole process of their development can be followed, from single rickettsiae through clumps consisting of only a few organisms, as are seen on the right side of the figure, to structures as large as those shown on its left side. Increasing further in size, the clumps merge into one another, giving rise, in some animals, to the voluminous rickettsial masses described previously.

The study of sections of lungs taken at various intervals after infection shows that the multiplication of extracellular rickettsiae is seen only at the terminal stages of rickettsial bronchopneumonia. It shows that extracellular rickettsiae, although always present in the infected lungs, are not increasing but diminishing in number up to the 48th hour, when, as has already been mentioned, individual rickettsiae begin to grow out from the infected cells. It is conjectured that the extracellular growth of rickettsiae, universally believed to develop only inside living cells, becomes possible in the lungs of rats and mice[5] at a moment when the substances indispensable to their multiplication and contained in the cells are released from injured cells.

The interpretation of the extracellular clumps as agglutinated rickettsiae can hardly be put in accordance with what is known about the time of development of the agglutinins. No animal whose lung has been used for this study has survived up to the 96th hour after infection, and the antibodies are known to appear in appreciable amount only about 1 week after the contact between the microbes and the host.

Granular Structures in the Life Cycle of the Typhus Virus

Since rickettsiae grow in the cells of the lungs of rats as granular bodies and aggregates or diffusely, the granular structures, which seem to correspond to the "morulae" described by Begg, Fulton, and van

den Ende [2] and also to the "intracellular globular masses" found by Wolbach, Todd, and Palfrey [6] in human typhus, cannot be considered as a specific and indispensable phase in the development of the typhus virus. This view is supported by the fact that granular bodies have never been seen in the yolk-sac membrane, in the tunicae of guinea-pigs so far examined in histologic sections, or in the epithelial cells of the gut of the louse, their natural medium.[6] Moreover, in the lungs of mice and rats granular bodies are never seen in bronchial epithelial cells in which rickettsiae are as numerous as in alveolar cells. In addition, the shape and structure of the granular bodies make it unlikely, in accordance with the observation of Begg and associates, that they play the same rôle in the development of the typhus virus as the "morulae" in the development of the psittacosis virus. Although fairly characteristic, the shape of the granular bodies is variable. Transitional stages exist between granular bodies and diffusely growing rickettsial masses which often merge into a fully characteristic granular body (Fig. 31). Granular bodies are not cystic structures filled with rickettsiae because they have no limiting membrane and also because they are not composed of masses of loose rickettsiae but, as shown in Figure 29, of cytoplasm stuffed with organisms. Finally, no disrupting or disrupted granular body has ever been seen in the evolution of typhus virus in the lungs of several hundred mice and rats so far examined. For these reasons the granular bodies cannot be considered as specific structures representing an obligatory phase in the development of rickettsiae, and the suggestion of the existence of a development cycle as proposed by Begg, Fulton, and van den Ende [2] cannot be accepted.

The granular bodies are regarded merely as colonies of rickettsiae. It has been demonstrated that they develop from the intracellular clumps observed 5 hours after infection, which are the earliest colonies or growing centers of rickettsiae. The fact that granular bodies develop in alveolar cells but are never seen in the cells of yolk-sac membranes, in the cells of the louse gut, or in bronchial epithelial cells, shows that the main factor in their development is the nature of the cells, the strain of rickettsiae used influencing only their number and size. Experiment shows, indeed, that every strain, whether murine or epidemic, gives rise to the development of granular bodies in the alveolar cells, but that the number and size of these structures vary with the strain.

SUMMARY

1. In rats, intranasal infection with typhus rickettsiae results in a bronchopneumonia strongly focal in character. The foci merge into one another and the inflammatory process thus extends progressively. In

the terminal stages large portions of lung are consolidated, the upper and central parts being usually the more severely involved, but not as regularly as in mice. The consolidation is neither so intense nor so extensive as in mice. Exudate is scanty but marked swelling and desquamation of the alveolar cells are prominent features.

2. It is considered that the desquamation and massive elimination of infected alveolar cells and individual rickettsiae are the expression of a strong, though not specific, reaction of the rat lung to typhus virus.

3. Rickettsiae are found in every stage of their development in the lung. Therefore, the existence of invisible forms of the typhus virus seems improbable.

4. In the rat lung, rickettsiae are progressively engulfed by the cells and at the same time undergo characteristic morphologic changes. Then they multiply by transverse fission like bacteria, develop granular bodies and irregularly shaped aggregates, or grow diffusely within the cells. Finally, individual rickettsiae grow out from the infected cells. These organisms may be taken up by other cells or may develop extracellularly, giving rise to extracellular clumps and voluminous rickettsial masses. The extracellular growth of rickettsiae is thus demonstrated.

5. The granular bodies are colonies or growing centers of rickettsiae. There is no evidence of their being a specific phase in a developmental cycle of the typhus virus.

REFERENCES

1. Chandler, A. C. Introduction to Parasitology, with Special Reference to the Parasites of Man. J. Wiley & Sons, New York, 1940, ed. 6.
2. Begg, A. M., Fulton, F., and van den Ende, M. Inclusion bodies in association with typhus rickettsiae. *J. Path. & Bact.*, 1944, 56, 109–113.
3. Bedson, S. P., and Bland, J. O. W. A morphological study of psittacosis virus, with the description of a development cycle. *Brit. J. Exper. Path.*, 1932, 13, 461–466.
4. Castañeda, M. R. Experimental pneumonia produced by typhus rickettsiae. *Am. J. Path.*, 1939, 15, 467–475.
5. Nyka, W. A histological study of the lungs of mice infected with typhus rickettsiae. *J. Path. & Bact.*, 1945, 57, 317–324.
6. Wolbach, S. B., Todd, J. L., and Palfrey, F. M. The Etiology and Pathology of Typhus, Being the Main Report of the Typhus Research Commission of the League of Red Cross Societies to Poland. Harvard University Press, Cambridge, 1922.

[*Illustrations follow*]

DESCRIPTION OF PLATES

PLATE 133

All rickettsiae were stained with methyl violet and metanil yellow. $\times$ 1250.

FIG. 1. Three minutes after infection. Minute dots and coccobacilli among the cells of a septum (top), and in the inoculum inside an alveolus (bottom).

FIG. 2. One hour after infection. Fine dots and tiny diplococci in the alveolar cells of a septum.

FIG. 3. Three hours after infection. Fine dots, coccobacilli, and rods, singly or in small groups.

FIG. 4. Five hours after infection. Two small clumps in the cells of a septum. Insert shows two diplobacilli in exudate.

FIG. 5. Seven hours after infection. Two clumps (above) and a single adult rod (below) in the cells of a septum.

FIG. 6. Nine hours after infection. Further development of the clumps.

FIG. 7. Twenty-four hours after infection. Clumps increased in number and size located in a septum.

2

3

6

5

7
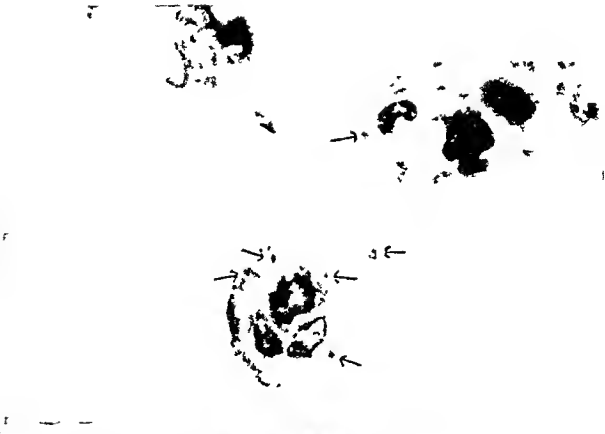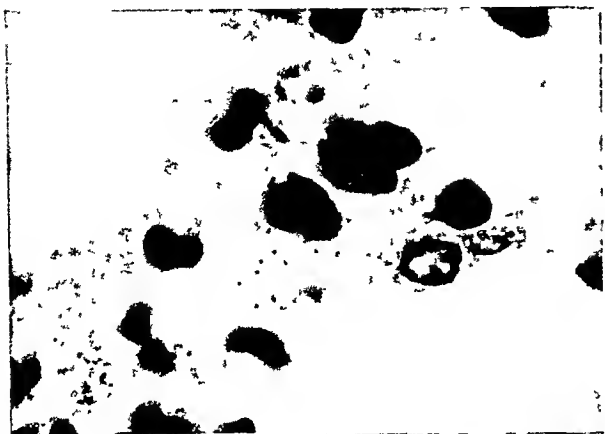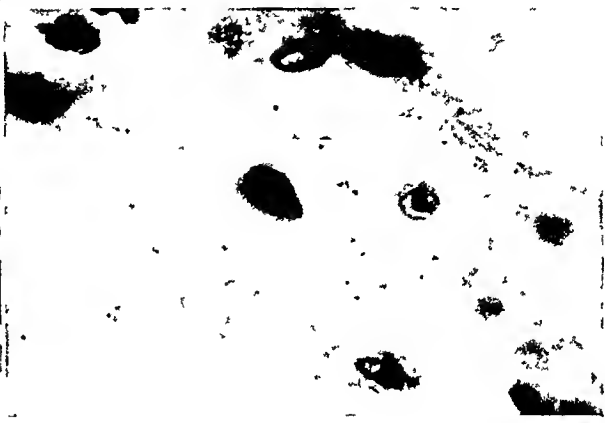
Nyka

Rickettsiae of Murine Typhus

PLATE 134

FIG. 8. Thirty-three hours after infection. Earliest granular bodies and a few clumps.

FIG. 9. Forty-eight hours after infection. A few granular bodies and several aggregates in a slightly thickened septum.

FIG. 10. Fifty-five hours after infection. Numerous, fairly large granular bodies and irregularly shaped aggregates within a thickened septum.

FIG. 11. Seventy-two hours after infection. Numerous full-grown, well defined, fairly regular granular bodies and irregular dense aggregates.

FIG. 12. Rat, dead 4 days after infection. Very numerous intracellular and extracellular rickettsiae in the consolidated parts of the lung.

FIG. 13. From the same animal as Figure 12. Fairly numerous rickettsiae among and in the alveolar cells of the slightly thickened septa in the nonconsolidated part of the lung.

8
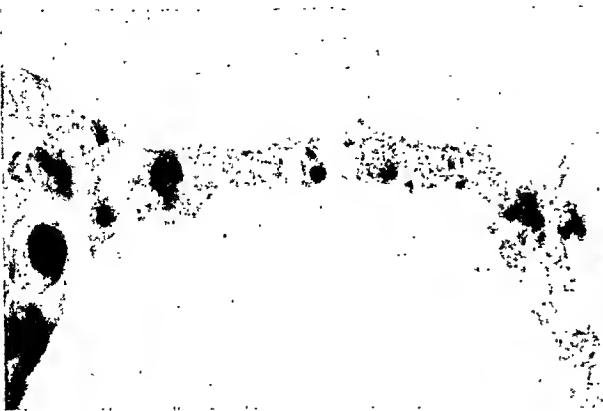
9

12

Nyka

Rickettsiae of Murine Typhus

PLATE 135
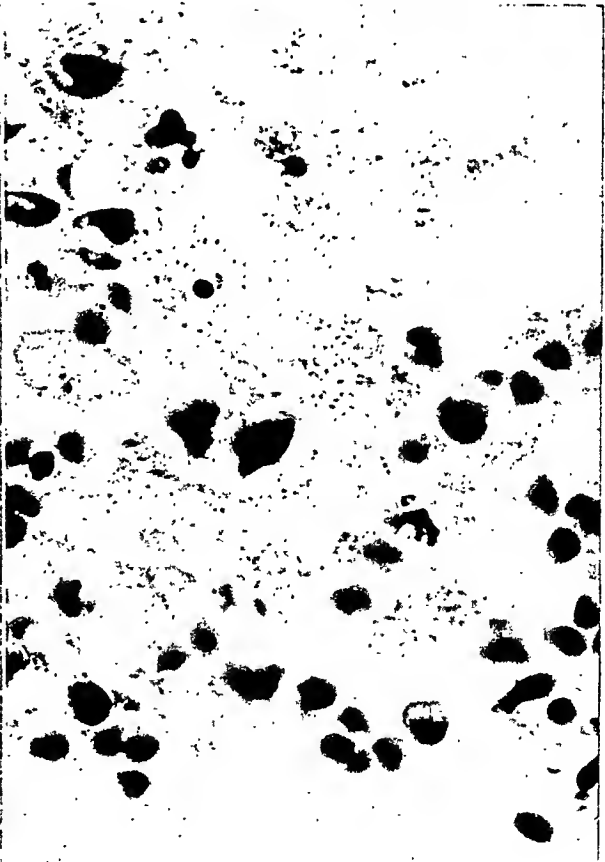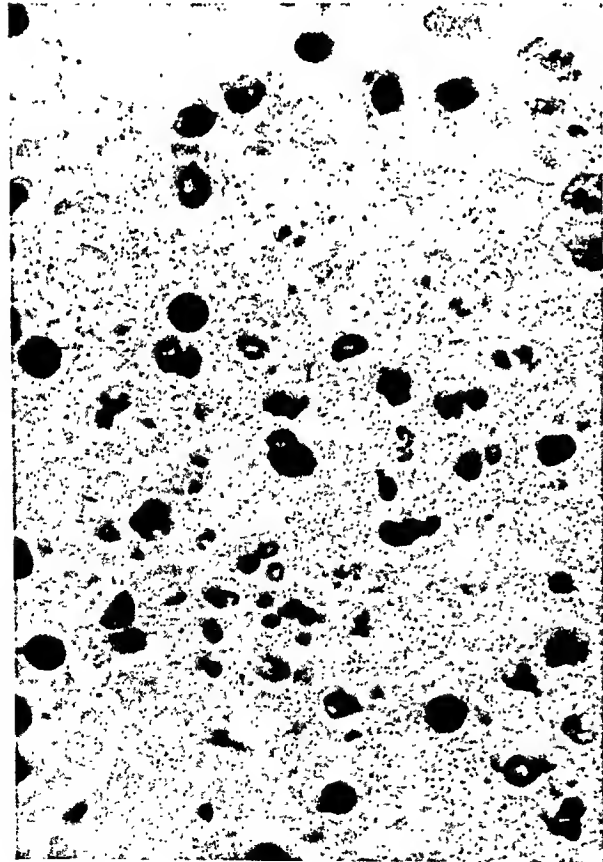
FIG. 14. From the same animal as Figures 12 and 13. Very numerous rickettsiae in the exudate filling an alveolus. Numerical preponderance of coccobacillary and rod types over the dot forms.

FIG. 15. From the same animal as Figures 12 to 14. Clumps of rickettsiae and single organisms in an alveolus filled with exudate.

(Figures 16 to 32 were taken from animals which died or were killed 3 or 4 days after infection.)

FIG. 16. Swollen alveolar cell with a few coccobacilli and rods in the cytoplasm.

FIG. 17. Swollen alveolar cell with a few dots and coccobacilli and a small, irregular, fairly dense aggregate in the peripheral part of the cytoplasm.

FIG. 18. Alveolar cell containing in the peripheral part of the cytoplasm some more or less dense, irregular aggregates of various sizes.

FIG. 19. Crescent-shaped aggregate of rickettsiae arranged around the nucleus of an alveolar cell.

FIGS. 20 and 21. Alveolar cells filled partly (Fig. 20) or entirely (Fig. 21) with diffusely growing rickettsiae.

FIG. 22. Alveolar cell with its cytoplasm filled with diffuse masses of rickettsiae and divided into a central and peripheral part by a clear line running parallel to the edge of the nucleus.

FIG. 23. Ring-shaped alveolar cell infected with rickettsiae arranged in the peripheral part of the cytoplasm.

FIG. 24. Irregular, fairly well defined, dense aggregate growing on the surface of a swollen alveolar cell.

FIG. 25. Alveolar cell with a few single rickettsiae inside the cytoplasm and a well defined, irregular. small aggregate on the surface of the cytoplasm.

FIG. 26. Alveolar cell with diffusely growing rickettsiae arranged in the peripheral part of the cytoplasm.

FIG. 27. Alveolar cell with cytoplasm stuffed with rickettsiae, remarkable for sharp outlines and regular shape, resembling a granular body.

FIG. 28. Alveolar cell with a handle-shaped nucleus (left) and cytoplasm transformed into a well defined and compact granular body.

FIG. 29. Voluminous granular body with a lateral protuberance studded with islets of rickettsiae-free cytoplasm.

FIG. 30. Alveolar cell containing a pyriform granular body and an irregular aggregate of rickettsiae.

FIG. 31. Alveolar cell filled with diffuse, homogeneous masses of rickettsiae merging into an oval granular body.

FIG. 32. Voluminous alveolar cell with cytoplasm filled with homogeneous, diffuse masses of rickettsiae from which single organisms grow out into the surrounding exudate.
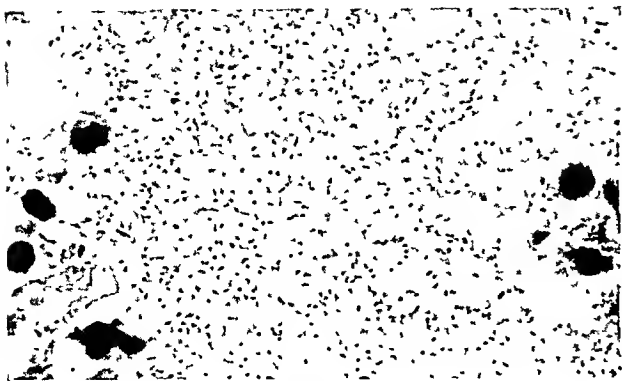
14

15

16

17

18

19

20

21

22

23

24

25

26

27

28

29

30

31

32

Nyka

Rickettsiae of Murine Typhus

FORTY-FOURTH ANNUAL MEETING

OF THE

AMERICAN ASSOCIATION OF PATHOLOGISTS

AND BACTERIOLOGISTS

CHICAGO

MAY SIXTEENTH AND SEVENTEENTH, 1947

THE AMERICAN ASSOCIATION OF PATHOLOGISTS AND BACTERIOLOGISTS

Forty-Fourth Annual Meeting, University
of Chicago, Chicago, Illinois
May Sixteenth and Seventeenth, 1947

PRESIDENT FORBUS IN THE CHAIR

BUSINESS MEETING

May Sixteenth, 1947

For the Council, the Secretary announced the following actions:
Election of new members

Charles P. Baker, Oakland, Calif.
Parker R. Beamer, St. Louis
Warren A. Bennett, Washington
William G. Bernhard, Summit, N.J.
Herman T. Blumenthal, Louisville
Warren L. Bostick, San Francisco
William H. Carnes, Baltimore
Jacob Churg, Paterson, N.J.
Jose Curiel, Mexico City
William L. Donohue, Toronto
Carl E. Duffy, Grosse Pointe, Mich.
Thelma B. Dunn, Bethesda, Md.
Patrick J. Fitzgerald, New York
Alfred Golden, Memphis
Clinton V. Hawn, Cooperstown, N.Y.
Elwyn L. Heller, Pittsburgh
Benjamin Highman, Silver Spring, Md.
Howard C. Hopps, Oklahoma City

Pao-chang Hou, Chengtu, Szechuan, China
Lalla Iverson, Durham, N.C.
Alfred G. Karlson, Rochester, Minn.
B. H. Kean, New York
Joseph F. Kuzma, Wauwatosa, Wis.
Thomas C. Laipply, Chicago
Frederick H. Lamp, Davenport, Iowa
Raffaele Lattes, New York
Stuart Lindsay, San Francisco
Ernst Loeffler, Chicago
Alfred M. Lucas, East Lansing, Mich.
Mark E. Maun, Detroit
Frank W. McKee, Rochester, N.Y.
Joseph F. A. McManus, Birmingham, Ala.
George Milles, Chicago
John Edgar Morison, Belfast, Ireland

Richard E. Olsen, Pontiac, Mich.
Lawrence Parsons, Reno, Nev.
Machteld E. Sano, Philadelphia
Edward C. H. Schmidt, Kansas City, Mo.
Ruell A. Sloan, Arlington, Va.

Edward B. Smith, Narberth, Pa.
Cyril Solomon, New York
Paul B. Szanto, Chicago
John L. Tullis, Bethesda, Md.
Lyle A. Weed, Rochester, Minn.
O. J. Wollenman, Jr., McKinney, Texas

Reinstatement to membership of Drs. Istvan A. Gaspar, Hugh G. Grady, and Preston Kyes.

Acceptance, with regret, of the resignations of Drs. James Miller, Max Pinner, and George Shanks.

The Council announced, with deep regret, the deaths of Drs. George T. Caldwell, Mortimer Cohn, L. U. Gardner, W. P. Larson, Emanuel Libman, Ward H. MacNeal, and H. E. Robertson.

Upon nomination of the Council, the Association voted to elect the following officers:

President	MALCOLM H. SOULE
Vice-President	E. W. GOODPASTURE
Secretary	HOWARD T. KARSNER
Treasurer	ALAN R. MORITZ
Incoming Member of Council	ROBERT A. MOORE

The Council announced that it had accepted the invitation of Dr. Virgil H. Moon to hold the next annual meeting of the Association at the Jefferson Medical College, Philadelphia, and that the meetings will be held on the Friday and Saturday preceding the meetings of the Federation of American Societies for Experimental Biology.

The Council announced that the topic for the symposium in 1948 will be "Diseases of Bones" and that Dr. Henry L. Jaffe will act as referee.

For the Council, the Secretary announced the re-election of Dr. Carl V. Weller as Editor-in-Chief of *The American Journal of Pathology* for the term of seven years; the re-election of Dr. Malcolm H. Soule as Assistant Editor of *The American Journal of Pathology* for the term of one year; the election of Dr. R. Philip Custer to the Editorial Board of *The American Journal of Pathology* for the term of seven years, to succeed Dr. J. Harold Austin whose term has expired.

The Secretary read a notice from Captain G. B. Ribble, U.S.N., in reference to Naval Reserve Medical Officers.

The Secretary read a letter from Dr. O. J. Pollak inviting attendance

at the organization of The American Society for the Study of Arterio-sclerosis.

The Secretary reported that the College of American Pathologists had requested appointment of two representatives of this Association to its Board of Directors. He stated that the Council had voted that participation as an association in the College of American Pathologists is considered to be outside the province of the Association. He explained that the Council had discussed this matter extensively before arriving at a conclusion. The attitude of the Council for many years has been that the Association has as its main functions the conduct of scientific meetings and of *The American Journal of Pathology*. The Council deemed it wise not to depart from this policy and took action without intimating prejudice for or against the College of American Pathologists.

SCIENTIFIC PROCEEDINGS

THE TISSUE ELEMENT IN THE ORIGIN OF NEOPLASMS. MORPHOLOGIC EVIDENCE THAT NEOPLASM ORIGIN IS PRIMARILY A TISSUE PHENOMENON. Anderson Nettleship, Detroit, Mich.

Abstract. Since there is still no accepted theory as to the mode of origin of cancer, a large number of human cancers in their earliest stages were studied in an attempt to determine if a common factor or factors were present to explain their derivation. In cases in which extremely early carcinomas were discovered, serial sections were made which included the whole neoplasm. The following types were examined: epidermoid carcinoma of the skin, epidermoid carcinoma of the cervix uteri, carcinoma *in situ* of the stomach, carcinoma *in situ* of the breast. In all cases it was possible to show that the tissue from which these neoplasms originated was in an involutionary phase and showed atrophic changes with scarring. It was also possible to demonstrate that the age of the cells in the early neoplasms, as nearly as could be determined morphologically, was the same in each neoplasm. No neoplasm was admitted to the group for study unless it showed the accepted criteria of cancer, including early invasion. Early basal cell carcinoma of the skin, melanoma, early carcinoma of the breast, and early carcinoma of the testis also were studied in order to clarify the occurrence of the asymmetrical unit formation in neoplasms. The occurrence of such units within the neoplasm, larger than the individual cell, was taken also as indicative of their multicellular origin. Cancer was shown in some instances to be limited to the area of known precancerous change, a rare probability if its origin was from a single cell.

Additional evidence was derived from sarcomas produced in tissue cultures treated with methylcholanthrene. The change from normal into cancerous cells always was found to be gradual; this is against the idea of a cell mutant being the origin of the cancer cell. On transplantation into animals, the tissue cultures produced sarcomas whose structure became increasingly anaplastic according to the length of time the original cultures had been exposed to methylcholanthrene.

The evidence strongly suggests that neoplasia comes about through the induction of cell groups at variously integrated levels: (1) widespread precancerous tissue atrophy, (2) carcinoma *in situ* with cells of identical age, (3) organization (asymmetrical) of neoplasms, and (4) induced sarcomas whose degree of anaplasticity depends upon the length of time the mother cultures were exposed to the carcinogen. Although the units are asymmetrical, abnormal cellular proliferation may occur at a number of different levels of tissue organization in tissues which are in an involutionary phase. No human cancer nor experimental cancer has ever been seen to originate from a single cell. If the above ideas are correct, research should be directed toward tissue phenomena rather than toward cellular phenomena.

THE SO-CALLED HÜRTHLE CELL TUMOR OF THE THYROID GLAND. J. B. Hazard and (by invitation) J. D. Ingle, Cleveland, Ohio.

Abstract. Eight cases of oxyphil-cell tumors of the thyroid gland were presented. Three were classified as carcinomas, five as adenomas. These tumors were of variable configuration. One patient with carcinoma died 6 months after the tumor was first observed clinically. Autopsy revealed metastases in the lung and right gluteal region. Death occurred in a second case 8 years after the tumor was first discovered and after two recurrences following operations. There were no metastases, although there was marked invasion locally. In a third instance the patient was alive and well 6 years after removal of the tumor. Of those with adenomas, two patients were alive at the end of 16 and 17 years, respectively; the others were

under observation for 1 to 3 years without evidence of recurrence. A ninth oxyphil tumor of the thyroid gland was presented with cells of parathyroid chief-cell type in the capsule and trabeculations. There was no clinical proof of parathyroid disease, but data were not available for complete evaluation. The histologic appearance of the oxyphil cells and the presence of chief cells strongly suggested parathyroid origin.

The oxyphil cells which are found occasionally in involuting thyroid tissue bear a similarity to cells of the so-called Hürthle cell tumors. Oxyphil cell neoplasms are of variable configuration and they may be benign or malignant, according to the circumstances of growth. Occasionally, oxyphil cells are intermingled with cells of undoubted thyroid origin in the same neoplasm. It is believed that the majority of the so-called Hürthle cell neoplasms originate in thyroid cells and as such should be designated as thyroid adenoma or adenocarcinoma, as the case may be, of oxyphil type. On present evidence there is an indication that these tumors, when malignant, may follow a slower course than thyroid adenocarcinoma generally and on this basis should be separately classified. In a rare instance, acidophil cell tumors of parathyroid origin may occur in the thyroid gland.

Discussion

(Dr. C. V. Weller, Ann Arbor, Mich.) It is fully in accord with the speaker's ideas that we note constantly in our Michigan material that the cells of adenomas which obviously are benign tend to acquire an oxyphilic character with the use of iodine for a long period. At the same time, the epithelium of the acini proper becomes atrophic, so that there is a reciprocal relationship. I believe fully in the thesis put forth that the Hürthle cells are not special cells, other than that they are adenoma cells influenced by the long-continued use of iodine, and may give origin to carcinomas which have similar characteristics. I wonder if anybody else has had occasion to relate this development to the use of iodine.

VERRUCOUS CARCINOMA OF THE ORAL CAVITY. Lauren V. Ackerman, Columbia, Mo.

Abstract. Verrucous carcinoma is a relatively infrequent type of neoplasm of the oral cavity, arising most frequently from the buccal mucosa and alveolar ridge of the mandible. It has a distinct tendency for papillary growth and presents considerable difficulty in diagnosis on biopsy. It spreads slowly over a wide area and tends to invade contiguous soft tissues, growing through the skin of the cheek, and into the mandible. In spite of extensive local invasion, regional lymph nodes are almost invariably spared. Its gross and microscopic characteristics are typical.

Discussion

(Dr. Harold L. Stewart, Bethesda, Md.) I should like to ask concerning possible etiologic factors in these cases. Khanolkar of Bombay, India, has reported a lesion somewhat resembling the lesion described, which he called "Chutta cancer." The natives smoke a local type of cigar, the "Chutta," and put the burning end in their mouths. They get cancer of the hard palate. I am curious to learn whether any similar etiologic factor was known in these cases of Dr. Ackerman.

(Dr. Ackerman) I am glad Dr. Stewart asked that question. We have been impressed by the fact that Missouri farmers frequently chew tobacco. In my series, 18 of the group of 31 chewed tobacco, and, as you recall, the majority of the lesions were in the region of the buccal mucosa where the chewing tobacco is usually kept. It is also interesting that with an average age of 67, there were 5 females, of whom one was 41, and this one, like some other Missouri farmers' wives, also chewed tobacco. Chewing of tobacco, therefore, may be of some etiologic significance. About one-third of these patients also had leukoplakia and a great many of them had very poor dental hygiene.

About a year ago I talked with a pathologist from the Tata Institute, Bombay, India. After looking at these lesions, he thought that they were not similar to any he had seen in India.

CANCER CELLS IN BRONCHIAL SECRETIONS. Peter A. Herbut, Philadelphia, Pa.

Abstract. In order to establish an earlier diagnosis in carcinoma of the lung, we have developed a method of examining bronchial secretions for neoplastic cells. In the course of an ordinary bronchoscopic examination, secretions are secured from the bronchus that drains the area containing the suspected cancer. If there are no secretions present, the area is washed with physiologic saline solution and the washings are aspirated. The material obtained is sent to the laboratory where thin smears are prepared by the Papanicolaou technic and examined for cancer cells. In most instances these can be precisely identified. In general, the diagnostic criteria consist of: (1) Changes in shape and size of cells. Normal epithelial cells are relatively small columnar, cuboidal, or occasionally round. The first two bear cilia. Cancer cells are not ciliated. They vary in size from normal to ten times the normal diameter, and they are of every conceivable shape. (2) Cytoplasmic alterations. Normally, the cytoplasm stains light green and is moderate in amount. Cancer cells show scanty to abundant pink, orange, gray, dense or granular cytoplasm. (3) Changes in the nuclei. Normally, these are round, oval, vesicular, and relatively small. Neoplastic cells have an increased nuclear-cytoplasmic ratio. The nuclei are round, oval, or extremely bizarre and irregular. They are either so intensely hyperchromatic that no internal structure can be discerned or they are very lightly stained and washed out. The latter are always large and have distinct borders, clumped nucleoplasm, and often prominent nucleoli.

To date we have examined 525 preparations. In this group there were 118 cases of cancer of the lung. A cytologic diagnosis of carcinoma by the method described was rendered in 105, or 89 per cent. In this same group of 118 cases it was possible to remove tumor bronchoscopically for histologic study in only 52 cases, or 44 per cent. In an additional 23 cases there was bronchial stenosis or distortion, but a tumor was not visualized. In 32 cases, or 27 per cent of the total in which cancer cells were found in the secretions, bronchoscopic examination was negative. A false positive diagnosis was rendered in only 4 cases.

As a result of this work our preoperative morphologic diagnoses of carcinoma have been doubled and our patients are being operated upon much sooner than was heretofore possible.

Discussion

(Dr. Jacob Werne, Jamaica, N.Y.) In those cases in which there was a discrepancy between the finding on biopsy and that by smear, what other evidence is there, either clinical or post-mortem, to substantiate the correctness of the diagnosis made after examination of the smear?

(Dr. William Boyd, Toronto, Ont.) A week ago I was in Montreal and I saw a demonstration by Dr. Mathews of the Montreal General Hospital in which the technic was a little different, but the results were certainly as striking. Mathews used sputum rather than bronchial secretions. He had previously used bronchoscopic secretion, but was more satisfied with sputum examination. His technic was to collect a 24-hour specimen of sputum, put it in a muslin bag, immerse it in Bouin's fixative, which caused it to contract greatly, and cut sections of the material. He stained them with hematoxylin and eosin, and the color photographs were quite dazzling in their beauty. I have never seen anything more striking. One glance enabled one to tell that these preparations were malignant. They were cases which had been proved by operation or autopsy to be carcinoma, and the sputum diagnoses were 64 per cent correct.

My last point is, what is the advantage of the Papanicolaou method? Dr. Mathews obtained equally good results with hematoxylin and eosin.

(Dr. B. Earl Clarke, Providence, R. I.) I believe there were 500 cases studied, and 118 were proved positive. Will Dr. Herbut please tell us the results of the other 382 cases? How many of these were positive by smear?

(Dr. William H. Harris, New Orleans, La.) Would Dr. Herbut kindly define and describe an "unmistakable cancer cell"?

(Unidentified discussant) May I inquire as to whether there were any errors in the diagnosis when a diagnosis of cancer was made, and how often these occurred?

(Dr. Herbut) I am not quite sure that I understood the first question. Every case in which a positive tissue specimen was secured bronchoscopically was found to be positive by the smear method. Is that what you mean?

(Dr. Werne) There was a discrepancy between the finding on biopsy and that by smear in some cases; what was the other clinical or post-mortem evidence to substantiate the correctness of the smear diagnosis?

(Dr. Herbut) In each one of these cases the diagnosis was made at thoracotomy and proved histologically. Every one of these 118 cases was a proved case, either by autopsy, by thoracotomy, or pneumonectomy; histologically, at any rate.

As far as sputum is concerned, I have not tried the method which Dr. Boyd outlined. In about 15 cases in which we were able to make a diagnosis on secretions obtained bronchoscopically, I did, however, examine the sputum faithfully, sometimes as many as a dozen different specimens, and in not one was I able to find a single cancer cell.

(Dr. Boyd) Did you examine them by section?

(Dr. Herbut) No.

As far as the Papanicolaou method is concerned, I think it has a distinct advantage. Squamous cell carcinoma is the most common type of cancer of the lung, and by this method one finds neoplastic cells in secretions staining pink, orange, or red. One glance at a smear will tell you it is carcinoma.

I said there were 525 preparations made. Naturally, many of the cases suspected of being carcinomatous turned out to be bronchiectasis, abscesses, tuberculosis, etc. These constituted the other 407 cases.

As to "unmistakable neoplastic cells," if you had studied these smears as we have, and in each one of these cases made smear preparations of the tumor, and compared the smears of the tumor with the smears obtained bronchoscopically, you would see that the cells are unmistakably neoplastic cells. One can superimpose one cell upon the other.

The errors in diagnosis: As you saw, our diagnosis was correct in 89 per cent of the cases. In 11 per cent we were unable to make a diagnosis by the smear method. That percentage has decreased, for in the last 60 cases we have missed only 3.

We have made only 4 over-diagnoses, and these were in the earliest part of the study. Three were cases of pulmonary abscess in which there was metaplasia of the epithelium. In each case there was only one nest of rather regular squamous cells. With added experience these offer no difficulty, for since then we have picked up several similar cases which we did not call neoplastic. In one case I made a diagnosis of carcinoma by finding a single cell, and when I look back at that now I do not think it was justified.

PRECANCEROUS LESIONS OF FORESTOMACH OF MICE INDUCED BY 20-METHYLCHOL-ANTHRENE AND 1,2,5,6-DIBENZANTHRACENE. Harold L. Stewart and (by invitation) Egon Lorenz, Bethesda, Md.

Abstract. Mice were given, instead of drinking water, an aqueous mineral oil emulsion containing 1,2,5,6-dibenzanthracene or 20-methylcholanthrene. *More than*

50 per cent of the animals developed squamous cell carcinoma of forestomach and precancerous lesions. The majority of the animals showed, singly or in combination, acanthosis of the squamous epithelium, hyperkeratosis, umbilicate foci with dyskeratosis and solitary or multiple papillomas. The areas of acanthosis and hyperkeratosis appeared as white, firm, corrugated projections from the mucosa, which were widespread, largely occluded the lumen of the viscus, and frequently showed dyskeratosis. At the keratinized margin overlying an area of dyskeratosis, there was usually a focal inflammatory area with a linear break in the keratin above. In this area there was loss of the granular cells and parakeratosis; some of the anuclear parakeratotic cell-like bodies were distended with fine acidophilic refractile granules, or were completely filled by deeply stained or pale acidophilic hyalin. These changes were observed in the umbilicate lesions and in the papillomas.

Carcinoma *in situ* was characterized by the presence of one, several, or all of the precancerous lesions described, spread over a wide segment of the forestomach and exhibiting multicentric development of carcinoma.

These specimens of forestomach showed diffuse and focal inflammatory cell infiltration of the lamina propria, the submucosa, and the subserous and intermuscular connective tissue, and, less frequently, the muscle coats. Amyloid was almost always present in the lamina propria of the forestomach, occurring in the walls of the vessels and being most marked in the tips of the papillary processes and in the cores of the papillomas. In contrast, the submucosa was almost always free of amyloid. Degenerative changes in the collagen and reticulum of the lamina propria and submucosa were regularly observed in association with the inception of dyskeratosis of the overlying epithelium.

Discussion

(Dr. Anderson Nettleship, Detroit, Mich.) I would like to ask in regard to the diet of these animals. It is well known that a vitamin-A deficiency produces the same type of lesion. Did these animals eat well? The second thing I would like to speak about is the widespread character and breakdown of these precancerous lesions. I would like to ask Dr. Stewart if he can tell about the time of occurrence of the atrophy and the precancerous lesion in their relationship to each other—which came first, or were they simultaneous, and how long did it take for the neoplastic changes to develop?

(Dr. Ruth Silberberg, St. Louis, Mo.) I would like to ask if there were any strain differences in the susceptibility to this type of tumor, and also whether there is any parallelism in the susceptibility to this type of tumor and to skin tumors produced by carcinogenic agents?

(Dr. Emmerich von Haam, Columbus, Ohio) Were there any metastases?

(Dr. Alfred Angrist, Jamaica, N.Y.) I wonder whether Dr. Stewart will tell us about the diet these animals were kept on. In a series of experiment some years ago, and there was an earlier description of this type of material on vitamin-A and B deficiency diets, this lesion occurred spontaneously in the forestomach of rats. It would be interesting to know whether the diet would have any effect as to the sequence in time of the lesion and the degree of its appearance and development.

(Dr. Stewart) We kept our mice on dog chow. These animals were toxic; they did not gain weight as well as the controls; however, it was possible to keep them living for as long as 13 months. They will develop anasarca if they are on the emulsion for a long time, and, interestingly enough, the anasarca disappears if they are taken off the emulsion regime and put on water. The diet is very important. I would not be surprised to find that they did have a vitamin A deficiency because of the mineral oil which they ingest. We have studied a number of animals on vitamin-A deficiency diets. A number of years ago, Andervont kept various strains of mice on vitamin-A deficient diets and, although we saw papillomas, we never saw a carcinoma of the forestomach, or precancerous lesions. In our con-

trol mice we saw hyperkeratosis and acanthosis, but in these there was no evidence of dyskeratosis, and no carcinoma developed. The question of the relation of the diet to this sort of thing is very interesting and requires experimentation.

The earliest tumor appeared at autopsy at 87 days, the earliest precancerous lesion at 78 days. Our experiment was not designed to determine just exactly when they appeared. It was designed to induce carcinoma and we tried to keep the mice living as long as possible to see whether metastases would occur. As a result of our work with 90 mice in this experiment, we are not prepared to say exactly when these lesions first develop. Of these 90 mice, 57 developed squamous cell carcinoma of the forestomach, and about the same number showed precancerous lesions. Some of the mice with carcinoma showed precancerous lesions in other parts of the forestomach. Forty-six per cent of the mice with carcinoma of the forestomach showed metastases, or local extension, to pancreas, spleen, peritoneum, diaphragm, regional lymph nodes, liver, kidney, genital omentum, and lungs.

There is a strain susceptibility. In the ABC backcross mice, we never obtained squamous cell carcinoma of the forestomach with olive oil emulsions of 1,2,5,6,-dibenzanthracene, and yet in strain A mice we obtained this lesion.

We have not investigated the possibility of any parallelism between induction of carcinoma of the forestomach and induced skin tumors.

The relationship between the precancerous changes and the atrophy of the mucosa of the forestomach requires further study.

THE DISTRIBUTION OF PARIETAL CELLS IN GASTRIC DISEASE. William A. Meissner, Boston, Mass.

Abstract. There is now general agreement that the hydrochloric acid of the stomach is secreted, directly or indirectly, by the parietal cells of the gastric mucosa. Although the determination of gastric acidity has become almost a routine procedure in the study of stomach disorders, particularly peptic ulcer and cancer, the state of the cells which secrete the acid has been left largely to conjecture.

An attempt was made to determine whether there are quantitative or qualitative differences in the parietal cells in conditions in which there is usually hyperacidity (peptic ulcer) as contrasted with conditions in which the acid is low or absent (gastric cancer). A series of 200 surgically resected stomachs, removed because of gastric cancer or peptic ulcer of the stomach or duodenum, was examined. Multiple sections were taken from each stomach, insofar as possible from the same representative areas, and the histologic appearance of the parietal cells was noted; likewise, an estimate was made as to whether the cells were abundant or moderate to few in number in each section. The parietal cells in all specimens diminished in number as the pylorus was approached and were fewer along the entire lesser curvature when contrasted with the opposite areas on the walls or greater curvature. The only quantitative change of significance was that many cases of carcinoma showed a marked diminution in the total number of parietal cells in the body and fundus, whereas they were diminished less frequently in these areas in ulcer. This diminution, however, was not a constant finding in cancer, and many cases with cancer and complete anacidity showed abundant parietal cells; no stomach showed a complete absence of such cells. As to qualitative changes in the individual cells, as seen in routine stains, there were no specific nuclear or cytoplasmic alterations which could be correlated with hyperacidity or anacidity.

The cause of diminished secretion of gastric acid cannot be explained alone on quantitative or qualitative morphologic changes in the parietal cells. Further work is warranted to determine such cause or causes since the problem is of more than academic interest. It has been frequently noted that anacidity may precede the onset of carcinoma of the stomach.

Discussion

(Dr. Howard C. Hopps, Oklahoma City, Okla.) I would like to know whether Dr. Meissner has observed gastric cancer in which the origin appears to be in the parietal cells, and, if so, what was the character of this neoplasm as far as acid secretion was concerned.

(Dr. Meissner) I have never observed in our material a carcinoma which could be definitely proved to arise from the parietal cells of the stomach. There are many gastric carcinomas with cells similar to parietal cells, but I believe in most instances they are merely degenerated tumor cells. Carcinoma cells often become acidophilic when they degenerate.

PRIMARY TUMORS OF THE PERITONEUM. Louisa E. Keasbey (by invitation), Los Angeles, Calif.

Abstract. No comprehensive review of tumors arising from the lining cells of the peritoneal cavity is to be found in the Anglo-American literature. These tumors are rare. Not only is misapprehension prevalent as to their structure, types, and clinical course, but there is incredulity as to their existence.

The purpose of this paper is to present a classification of the primary tumors of the peritoneum with a brief review of the literature and a report of 8 representative cases.

I. Primary carcinoma of the peritoneum (4 cases, one of the tunica vaginalis testis).

A. The papillary ascitic type (2 cases): This tumor begins as small nodules (which were seen and excised at laparotomy), sparsely scattered over the peritoneal surface. This is followed by a period of extreme recurring ascites. Later the peritoneal cavity becomes obliterated and ascites disappears. The tumor tends to spread over the entire surface of the peritoneum and at necropsy the peritoneal cavity is often found totally obliterated with such dense adhesions that no cleavage lines can be found. The tumor replaces no organ, does not metastasize, and is distinguished by limiting itself to uniform superficial invasion. Its histologic picture is considered distinctive.

B. The pseudomucinous type (1 case): Tumors of this type are said to present a more glandular structure than those of type A, but in the case reported this was not striking. The course of the disease and the microscopic and gross pictures are similar to those seen in type A, but the abundant peritoneal fluid is thick and mucinous or pseudomucinous.

C. The multicystic ascitic type: a representative case is discussed, but it is felt that these tumors are probably of retroperitoneal lymphangiomatous nature and are neither carcinomas nor of primary peritoneal origin.

II. Benign tumors of the peritoneum.

A. Primary peritoneal papillomatosis (2 cases, one involving the abdomen, but studied only at laparotomy; one involving the abdominal peritoneum, pleura, and tunica vaginalis testis).

B. Mesothelial adenoma (2 cases): Small benign serosal tumors, described independently in this country and in Italy as involving the serosal surfaces of the genitalia.

III. Primary tumors of the tunica vaginalis testis—an extension of the peritoneum (3 cases: carcinoma, papilloma, and adenoma).

THE BENIGN GIANT CELL TUMOR OF TENDON SHEATHS. AN EXAMPLE OF SCLEROSING HEMANGIOMA.* Lee N. Foster (by invitation), Boston, Mass.

Abstract. The present study reviews 41 tumors emphasizing sequential tissue changes. Most striking of these is a progressive increase in fibroblastic stroma.

* *Am. J. Path.,* 1947, 23, 567-583.

Specimens in which little fibrosis is evident are highly vascular, the blood vessels being separated from one another by a cellular tissue. As stromal overgrowth destroys this vascular pattern, intravascular cells phagocytose lipid and hemosiderin. Despite regressive features induced by sclerosis, mitotic figures appear in the intervascular cells, defining the lesion as a true tumor. The activities of the intervascular cells as well as the manner in which the sclerosing process is centered about blood vessels suggest that the lesion is a sclerosing hemangioma.

Discussion

(Dr. D. Murray Angevine, Madison, Wis.) I should like to point out that the sub-synovial tissue of the tendon sheath is a very vascular tissue. I would like to ask Dr. Foster if he has ever seen an angioma of the tendon sheath. They frequently occur around joints. I would like to be sure that angiomata occurred before we classify these lesions as sclerosing angioma. We do have angiomata in the skin and that is the place where sclerosing angiomata occur.

(Dr. Alfred S. Giordano, South Bend, Ind.) I should like to ask whether Dr. Foster has ever seen a giant cell tumor such as that demonstrated in a sclerosing hemangioma of the skin.

(Dr. Louis Lichtenstein, New York, N.Y.) We have been aware for some time of the thesis entertained by Dr. Foster and his colleagues, and I followed his presentation with a great deal of interest. Frankly, I am no more convinced of its soundness now than I was before. This lesion is not unique in the tendon sheath, and has its anatomic counterpart in the synovial lining and sub-lining connective tissue of joints, and also of bursae. Some years ago Drs. Jaffe, Sutro, and I described these lesions with the idea that we were dealing with some peculiar inflammatory condition which we designated pigmented villonodular synovitis and bursitis, referring to its tendon sheath expression as pigmented nodular tenosynovitis. Thus, the nodules springing from the modified synovium in cases of pigmented villonodular synovitis may be indistinguishable histologically from the tendon sheath nodules in question. The early stages of evolution of the condition can be observed to better advantage in the synovial and bursal lesions than in the tendon sheath nodules, since the latter are generally far along in their development when they are extirpated, and already show more or less extensive fibrosis. If one examines the early stages histologically, one observes, to be sure, that the lesion is rather vascular, but its most striking feature is intense proliferation of large polyhedral cells, which may be so compact as to suggest a neoplasm. These cells, however, are macrophages by function, and they take up hemosiderin pigment granules and, in some cases, lipids as well. Eventually they tend to be replaced by collagenized fibrous connective tissue. Now, some of these macrophages appear to be derived from the adventitial cells of blood vessels, but if that establishes the nature of the lesion as a hemangioma, then, to be consistent, one would have to call practically any inflammatory lesion a hemangioma.

(Dr. Helen Ingleby, Philadelphia, Pa.) Have any of the tumors been stained for reticulin? If characteristic reticulin formation was present in the earlier tumors, it might help clarify the etiology.

(Dr. Foster) In answer to Dr. Angevine's question regarding the relationship of such lesions to synovial membranes, this question has been partially answered by Dr. Lichtenstein. Such lesions do occur in relationship to the synovial membrane.

In answer to Dr. Giordano's question, I have seen such giant cell tumors in the skin.

In answer to Dr. Lichtenstein's discussion, I believe our observations are quite similar, but the discrepancy arises in our opinion as to whether these are progressive growths. In studying these tumors I have felt I could find evidence of continuous growth throughout the sequences of sclerosis, which led me to believe

that the lesion was a true tumor. He apparently has not observed this, or has not felt it important in interpreting the nature of the lesion, and so for this reason I believe we diverge in our final conclusions.

A New Form of Granulomatous Disease in Man Characterized by Intra-cellular Parasitism Due to an as Yet Unidentified Acid-Fast Organism. J. T. Cuttino, A. McCabe, and M. L. Weil, Jr. (by invitation), Durham, N.C.

Abstract. The problem considered in this report was presented by a 34-months-old white girl whose illness began 4½ months prior to death. A mass in the abdomen was diagnosed lymphosarcoma. Biopsy of an enlarged node showed many large macrophages containing great masses of acid-fast organisms. The infant died 2½ months later after a course resembling inanition. At autopsy there was massive enlargement of the lymph nodes of the mesenteric and retroperitoneal groups with moderate enlargement of the left subclavian and mediastinal nodes, enlarged spleen, and ulcers of the colon. Microscopically, this enlargement was due to great proliferation of macrophages which contained many acid-fast organisms. There were additional foci of these parasitized macrophages in the liver, lungs, and pancreas. The organism was obtained in pure culture from the lymph nodes and spleen. It is pathogenic for, but not lethal to, guinea-pigs, mice, and rats. It is nonpathogenic for rabbits, frogs, chickens, and goldfish. Infection is uniformly successful by subcutaneous, intraperitoneal, and intravenous injection; by ingestion; and by instillation in the eye. The lesions produced consist of a central zone of necrosis surrounded by a broad zone of epithelioid cells. In about 10 per cent of instances infection becomes systemic, but intracellular parasitism is not as marked in experimental animals as in the human being. Organisms can be reclaimed in pure culture from these lesions. There is spontaneous healing, and a tuberculin type of sensitivity is produced. Morphologically, the organism is a gram-positive, strongly acid-fast, short filamentous, branching organism. Culturally, it produces a yellowish, moist colony in 3 to 5 days on Petragnini, Bordet-Gengou, and other media including Sabouraud's, at room temperature. On van Tieghum cell mounts, mycelia and branching appeared in 2 to 5 days. There are no aerial mycelia. This organism appears to be an actinomycete, specifically a Nocardia. However, no description of an identical organism has thus far been encountered. The histologic reaction is unusual in that it is entirely macrophagic. The intracellular parasitism is most closely allied to that of leprosy and Johne's disease of cattle, but in these diseases there are other reacting elements.

Discussion

(Dr. Roger D. Baker, Birmingham, Ala.) I should like to ask how this case differs from reported cases of nocardiosis or streptothricosis.

(Dr. Anderson Nettleship, Detroit, Mich.) I wonder if Dr. Cuttino could find any difference between this and Johne's disease? You can hardly see these macrophages with the punched-out central hole without thinking of Johne's disease of cattle. I wonder if he tried to prove it to be Johne's disease, since that would be the logical thing. I suppose the child lived in the country. Was there any evidence of Johne's disease in the cattle in the region from which she came?

(Dr. Cuttino) The lesion of nocardiosis is of a more purulent type, and in some species there is granule formation. In our human case, necrosis was not a feature. In experimental animals the lesion is somewhat similar, but shows extensive necrosis.

In reply to Dr. Nettleship, we have had opportunities to compare this with Johne's disease by means of sections we obtained from the Bureau of Animal Industry. In those cases the lesion was confined to the gastrointestinal tract, and there was not this massive display of organisms. The organisms were smaller,

and we have used a culture of the organism of Johne's disease, also obtained from the Bureau of Animal Industry, and were unable to produce the lesion in experimental animals.

MODIFICATIONS OF TUBERCULOUS LESIONS IN PATIENTS TREATED WITH STREPTO-MYCIN. Curtis M. Flory and J. W. Correll (by invitation), and J. G. Kidd, New York, N.Y.

Abstract. Five patients with miliary tuberculosis who had been intensively treated with streptomycin were examined post-mortem. In all cases there was evidence that the tuberculous lesions had been modified by the therapy.

In one patient, for example, extensive miliary tuberculosis of the lungs, demonstrated roentgenologically, had resolved completely after about 35 days of therapy; an associated tuberculous meningitis at first had responded to the drug given intrathecally but eventually had relapsed, causing an internal hydrocephalus and death 297 days after the initial diagnosis had been made. Gross examination of the lungs post-mortem revealed no trace of the miliary lesions, and cultures of the lungs for acid-fast bacteria were negative, though a healed primary complex was found. Microscopically, there were numerous small scars consisting of loose fibrous tissue scattered irregularly throughout all lobes of the lungs; these revealed no evidence of active tuberculosis, and they were interpreted as being the healed remains of miliary nodules. Similar scars were found post-mortem in the lungs of 3 additional patients who had responded temporarily to the therapy, though in these cases there was widespread recurrence of active tuberculosis along with focal fibrosis throughout the lungs.

In still another patient, whose miliary tubercles had disappeared following the initiation of drug therapy, as indicated by x-ray examinations, but had reappeared later when the infecting bacterium had become drug-fast, very many large miliary tubercles were visible in the lungs on post-mortem examination. Microscopically, these lesions were discrete and spherical, and each was composed of a thick fibrous capsule arranged concentrically about an area of granulomatous inflammation and caseation that often had broken through the fibrous barrier and more or less surrounded the latter. These lesions contained great numbers of acid-fast rods, as special stains revealed, and their "inside-out" character suggested that the bacteria responsible for them had been held in check for a time and then gained overwhelming impetus, perhaps when they had become resistant to the drug.

Post-mortem studies of the central nervous system in these 5 patients are still under way in the laboratory of Dr. Lewis D. Stevenson. The findings thus far, however, make it plain that the tuberculous lesions in at least 3 of the patients had been modified by streptomycin therapy.

STREPTOMYCIN-RESISTANT TUBERCLE BACILLI: EFFECT OF RESISTANCE ON THERA-PEUTIC RESULTS. William H. Feldman, A. G. Karlson (by invitation), and H. Corwin Hinshaw, Rochester, Minn.

Abstract. Objective: To determine if infections produced by tubercle bacilli having a marked *in vitro* resistance to streptomycin would respond to streptomycin therapy.

Procedure: Tubercle bacilli with normal sensitivity to streptomycin were obtained from a patient before treatment with streptomycin was started. A culture resistant to streptomycin *in vitro* was obtained from the same patient after treatment for 4 months with streptomycin. Two experiments were done concurrently. In one, guinea-pigs were infected with the sensitive culture and in the other experiment a similar group of guinea-pigs was infected with the resistant culture. Twenty days after inoculation 10 animals in each experiment were started on treatment with streptomycin. Treatment was continued daily until all of the untreated controls had died (approximately 23 weeks).

Results: The disease in the animals infected with the streptomycin-sensitive culture responded favorably to therapy. However, in 3 of 10 animals active lesions of recent origin were present. Streptomycin-resistant tubercle bacilli were obtained from each of these 3 animals. The disease in the animals infected with the streptomycin-resistant culture failed to yield to therapy. In this instance the amount and character of the tuberculosis in the untreated controls and in the treated groups were comparable.

Conclusions: Infections in guinea-pigs induced by tubercle bacilli resistant *in vitro* to streptomycin are refractory to therapy with this antibiotic.

Discussion of. Papers by Drs. Flory, Correll, and Kidd; and by Feldman, Karlson, and Hinshaw

(Dr. Richard H. Follis, Jr., Baltimore, Md.) I should like to ask what happened to the visceral lesions, that is, the lesions in the spleen, liver, and bone marrow, of the patients studied with miliary tuberculosis.

(Dr. M. H. Soule, Ann Arbor, Mich.) I would like to ask if there are any colonial or tinctorial differences, or differences in virulence that go hand in hand with resistance or sensitiveness to streptomycin.

(Dr. J. W. Correll) As far as visceral lesions in these patients were concerned, in a few cases we did find small hyaline scars in the liver, such as have been described by Dr. Baggenstoss. In one patient we found very fibrotic lesions in the kidneys. However, in other cases we found no definite evidence of modification.

(Dr. Feldman) In our experience, Dr. Soule, we have been unable to detect any significant colonial or tinctorial differences in the strains that were resistant or highly susceptible to streptomycin. From the standpoint of virulence it is my impression that possibly a diminution in virulence is associated with a state of resistance. However, we have little factual evidence on this question.

DEGENERATION AND NECROSIS OF NEURONS IN EIGHTH CRANIAL NUCLEI CAUSED BY STREPTOMYCIN. Lewis D. Stevenson, E. C. Alvord, Jr., and J. W. Correll (by invitation), New York, N.Y.

Abstract. Liquefaction necrosis of neurons, sometimes with dropping out of cells, was found post-mortem in the ventral cochlear nuclei in 5 patients who became partially or completely deaf while receiving large amounts of streptomycin for the treatment of tuberculosis. Similar changes were present in the inferior vestibular nuclei in 1 of 2 patients in whom these structures were studied. Other cranial nerve nuclei were normal in all cases. While all of the patients died with tuberculosis and all manifested varying degrees of tuberculous involvement of the cranial nervous system, there was no clinical evidence that the function of cranial nerves other than the 8th had been disturbed in any case. In one there was softening of a part of the basis of the pons immediately below the ventral cochlear nucleus, this change being unilateral, whereas the neuronal degeneration presumably due to streptomycin was bilateral.

Three dogs were given large doses of streptomycin and developed marked weakness and incoordination, although no deafness was noted. One died on the 9th day with necrotizing renal arteriolitis and glomerulitis, and the other two were sacrificed on the 28th day. All showed lesions in the ventral cochlear nuclei bilaterally, with liquefaction and neurons similar to that found in the clinical cases, but no apparent dropping out of cells. In the dog that died of renal disease, peculiar clumps of Nissl-like material were present in the cytoplasm of the cells of the ventral cochlear nuclei.

Apparently, streptomycin can cause in man and experimental animals a specific destructive effect on the neurons of the 8th cranial nuclei, especially the ventral cochlear nuclei, and possibly the inferior vestibular nuclei. These changes would

seem to be sufficient in some of the clinical cases to account for deafness and perhaps for vestibular dysfunction also.

Discussion

(Dr. Virgil H. Cornell, Washington, D.C.) I would like to ask whether any of these five patients demonstrated allergy to the drug during their early treatment and had to be desensitized. I ask this because of information transmitted to me by Dr. Romansky, and I wish to give him full credit for it. I understand that he has found the percentage of patients with 8th nerve injury to be very high following the use of streptomycin in tuberculosis. However, none of five patients, who had been allergic to the drug and whom he therefore had to desensitize before treatment was begun, showed any of these symptoms. I think it is a most important observation; it is one, I am sure, that he is going to follow up in the future, but I felt it was important enough to bring before this society for consideration.

(Dr. Cecil A. Krakower, Chicago, Ill.) I should like to ask whether the authors examined the cochlear apparatus or the semicircular canals in this connection, since we know that certain drugs can affect the end-organs in the internal ear.

(Dr. Archie Baggenstoss, Rochester, Minn.) I should like to ask if determinations were made of the streptomycin content of the brain. We reported two cases last year in which the cerebrospinal fluid became negative, and at autopsy no cerebrospinal meningitis was found, yet extensive tuberculous lesions were found in the brain. In one case the brain was analyzed for streptomycin content, and absolutely none was found.

(Dr. Alvord) In regard to the question of allergy, only one patient showed any signs of allergy, a fairly marked eosinophilia. Neither he nor any of the others was desensitized.

Concerning the question of the examination of the cochlear apparatus, we have not examined that; but as to the possibility of this being a retrograde damage, I would like to point out that the cells in the ventral cochlear nucleus are secondary neurons, and not those that supply the fibers in the 8th nerve or those to the internal ear.

As to streptomycin concentration, we have not analyzed that factor either.

THE INFLUENCE OF 3,3'-METHYLENE-BIS-(4-HYDROXYCOUMARIN) UPON STREPTO-COCCUS INFECTION IN RABBITS. George R. Thuerer (by invitation) and D. Murray Angevine, Madison, Wis.

Abstract. Prothrombin levels were determined on rabbits prior to and following the oral administration of dicoumarol. There was considerable variation in the response of the animals to dicoumarol. Some were entirely resistant, whereas the prothrombin time of those that responded was 3 or more minutes in contrast to the normal time of 4 to 7 seconds. These were paired with normal rabbits and both groups were infected with a single intracutaneous injection of virulent *Streptococcus haemolyticus*. The local lesions were measured daily, prothrombin levels were determined during the course of the infection, and blood cultures made when an animal died or was killed.

The skin lesions on the dicoumarolized animals were, with few exceptions, larger, more diffuse and more spreading than those of the controls. Some of the animals were killed at intervals after infection whereas others were followed until the animal died or the infection subsided. Of 14 rabbits that responded to dicoumarol, 7 developed a positive blood culture, 5 of them dying of septicemia. Only one of 15 control animals developed septicemia.

Histologic examinations were made of the skin lesions and draining lymph nodes of 10 treated and 10 control rabbits to determine and compare the amount of fibrin in the two groups. There was definitely more fibrin in sections from the control

group. Dicoumarol-treated animals that failed to respond by prolonged prothrombin time showed fibrin in similar amounts to the controls.

These experiments strongly indicate that the lack of fibrin formation due to an interference with the mechanism of coagulation in dicoumarolized animals may play a significant rôle in the more extensive spread and greater invasiveness of the infection in treated, as in contrast to control, animals.

Discussion

(Dr. Richard H. Follis, Jr., Baltimore, Md.) I should like to ask if this was a fibrinolytic strain, and suggest that more clear-cut observations might be made if a nonfibrinolytic organism was used.

(Dr. Angevine) This is a fibrinolytic strain, and is very virulent. We plan to use other organisms, but I believe it is more important to use other species of organisms first than to use other strains of streptococcus.

THE NORMAL HUMAN ADRENAL CORTEX AND ITS RESPONSE TO ACUTE DISEASE. Norman Zamcheck (by invitation), Boston, Mass.

Abstract. "Post-mortem autolysis" does not account for most of the changes found in the human adrenal cortex at autopsy. A systematic review of large numbers of cases has shown that histologically "normal" adrenal cortices are found when normal persons are killed instantly by violence. Characteristic and readily recognizable histologic changes are found in the adrenal cortices of patients dying of a variety of acute conditions, including infections, traumatic injury, poisoning, burns, and others. The pathogenesis of these changes as found in a group of men dying of diphtheria is presented.

THE PATHOLOGY OF ADDISON'S DISEASE: ADRENOCORTICAL CONTRACTION. Nathan B. Friedman, Washington, D.C.

Abstract. Between December, 1941, and December, 1946, pathologic material from 15 patients who exhibited adrenocortical contraction at autopsy was received at the Army Institute of Pathology. During the same period specimens from only 10 patients with Addison's disease caused by tuberculosis were accessioned. Prior to World War II, only 6 cases of adrenocortical contraction had been studied at the Institute, as contrasted with 21 cases of tuberculous Addison's disease.

The clinical syndrome was in many instances so atypical and confusing that the diagnosis of adrenal insufficiency was not entertained, particularly when the disease ran a short, fulminating course. Acute gastro-enteritis, poisoning, ruptured peptic ulcer, coronary occlusion, psychosis, intracranial hemorrhage, and myasthenia gravis were all simulated. The danger of temporizing with the medical emergency of adrenal insufficiency was underlined by the repeated occurrence of sudden collapse and death.

The morphologic picture in the 21 cases of full-blown adrenocortical contraction ranged from that of pure destructive atrophy, or collapse, of the cortex to that in which the regeneration of cortical cells and nodules in atypical patterns overshadowed and masked the underlying atrophy. Although the early stage of "atrophy" is rarely encountered in material obtained at autopsy, the Institute files contain 4 examples of cortical degeneration, necrosis, and inflammation so pronounced that one could conceive of their being the precursors of adrenocortical contraction.

The lesions in adrenocortical contraction differ strikingly from those caused by vascular occlusion, syphilis, and tuberculosis. They bear a strong resemblance to the lesions of necrotizing hepatic injury and its sequelae.

Discussion of Papers by Drs. Zamcheck and Friedman

(Dr. Sol Roy Rosenthal, Chicago, Ill.) In a study of the adrenal gland in chronic diseases such as pulmonary tuberculosis, I noted the changes described by

Dr. Zamcheck plus a more advanced stage of hyperplasia to form nodes in the cortex, especially the capsule. In 71 cases drawn from the Bruns General Hospital and the Army Institute of Pathology, it was found that there were 1.18 nodules per case in comparison to 0.38 nodule per case in those dying from traumatic sudden death. The average length of the disease in the cases studied was 9.5 months.

(Dr. William Boyd, Toronto, Ont.) I noticed that in one of the slides which Dr. Friedman showed he had two cases of unilateral contraction. These were from cases of Addison's disease. I wonder what the other adrenal was like.

(Dr. Howard T. Karsner, Cleveland, Ohio) How does the Army Institute of Pathology make a diagnosis of syphilis of the adrenal?

(Dr. Averill A. Liebow, New Haven, Conn.) Has Dr. Friedman noticed any inclusion bodies in the adrenals?

(Dr. Russell L. Holman, New Orleans, La.) I would like to ask Dr. Friedman whether any of the patients had a history of an acute episode which might have been associated with necrosis of the adrenal. There are reports of clinical cases of Waterhouse-Friderichsen's syndrome which recovered following the use of adrenal cortical hormone. Do you have such a history in any of the cases of "contraction" of the adrenal?

(Dr. Howard C. Hopps, Oklahoma City, Okla.) I should like to ask Dr. Zamcheck whether he believes that these changes in the adrenal cortex in relation to so many conditions play a direct rôle in the peripheral vascular collapse that so often characterizes the disease.

(Dr. N. Goormaghtigh, Ghent; Belgium) It is a great pleasure for me to hear Dr. Zamcheck's work. I made similar observations about 20 years ago, by studying material in the first World War. The reason why I have not continued to be interested in this problem is that so many factors influence the structure and the behavior of the adrenal cortex, and I think that it is a work for the future to dissociate the different factors which act on the suprarenal cortex.

(Dr. Zamcheck) Dr. Goormaghtigh has already indicated the principal difficulty in answering Dr. Hopps' question: the relationship between these adrenal changes and circulatory collapse cannot be fully understood until the rôle of the several other factors known or thought to be influenced by adrenal cortical function has been elucidated. Some of these are the following: salt and water metabolism, protein and carbohydrate metabolism, possibly control of serum-antibody and blood-lymphocyte levels, as well as maintenance of blood pressure. Changes in all or several of these variables occur simultaneously in the many acute diseases in which these histologic patterns were found, vascular collapse being the only one which is easily recognized clinically. It is certainly true that peripheral circulatory collapse was recorded in a very high percentage of the cases reviewed; and Rich emphasized the correlation between such changes and circulatory collapse in instances of death from overwhelming infection. But he has also pointed out that lesions of this type were not found in cases of death from postoperative shock in the absence of overwhelming infection.

The problem of attributing specific physiologic significance to histologic or cytologic alterations of the adrenal cortex is one that has challenged the efforts of several investigators. The magnitude of this problem becomes apparent when one realizes that adrenal changes occur not only in the many acute diseases described today but also in chronic disease, as for example in the endocrinopathies, hypertension, and possibly some malignancies; also differences of sex, age, and metabolic state, such as puberty, pregnancy, and menopause, may also be associated with recognizable differences in adrenal cortical cyto-architecture.

In order for such physiologic-pathologic correlations to be made, abundant post-mortem or biopsy material must be available from patients who were studied

exhaustively before death. These must be compared with normal controls obtained from violent deaths. Finally, supplemental animal investigations are needed to fill gaps not otherwise bridged. Such studies are in progress.

(Dr. Friedman) In answer to Dr. Boyd's question about unilateral contraction, the other adrenal gland had not been found at autopsy and it was assumed that it had been so markedly contracted that it could not be located.

With regard to syphilis of the adrenal, Dr. Karsner, I will be pleased to have you look at the sections the next time you are in Washington. The patient was being treated with penicillin for a frank taboparesis, serologically proved, and died at the end of the series of treatments, of adrenal insufficiency. At autopsy, lesions showing sclerosing destruction of the parenchyma, considered compatible with syphilis, were found. Only Zenker-fixed tissue was available, so that the Warthin stain could not be done. Possibly we could not have demonstrated spirochetes in view of the treatment.

With regard to Dr. Liebow's question, inclusions have been seen by other workers in some of these lesions. I did not see any, but if viruses can produce comparable lesions in the liver, there is no reason why they cannot in the adrenal.

As far as Dr. Holman's question is concerned, some of these patients gave a history of difficulty following a severe infection. Hemorrhagic lesions, as in the Waterhouse-Friderichsen syndrome, could culminate in contraction, but there has been no evidence that I know of to that effect. I am willing to accept the idea that if the adrenal lesions associated with infection are widespread and severe, and if the patient lives long enough, he might die of Addison's disease due to adrenocortical contraction.

EXPERIMENTAL THIAMINE DEFICIENCY IN THE RHESUS MONKEY. James F. Rinehart and (by invitation) Louis D. Greenberg and Melvin Friedman, San Francisco, Calif.

Abstract. Recent progress in nutrition has made possible the study of single deficiencies in the monkey. It seemed most timely to explore systematically the deficiency states in a primate whose metabolic processes might be expected to approximate most closely those of man. This report is concerned with studies of thiamine deficiency. Seven Rhesus monkeys were subjected to one or more episodes of acute thiamine depletion. It is clear that significant metabolic inadequacies precede demonstrable structural changes. Diminished food consumption and weight loss were manifest about 2 weeks after thiamine was removed from the diet. The blood thiamine at that time was in the range of 4 γ per 100 ml. or less which we believe represents an inadequate content for normal metabolism. As the deficiency is prolonged the animals become apathetic, inactive, and progressively weaker. This is followed by ataxia; at times, ptosis and tremor. Retching was observed in several instances. Even in such advanced states of depletion, administration of thiamine will produce dramatic improvement in locomotion, appetite, and reactivity.

Neuropathologic Findings. The most striking and perhaps most significant lesions were found in the nuclear structures of the central nervous system. While lesions were found in all animals, they were more extensive and severe in those subjected to two or more episodes of depletion. Of the 7 animals studied, bilateral symmetrical foci of degeneration were observed most commonly in the putamen (5), caudate nucleus (4), inferior colliculi (4), cerebellar vermix (4), and certain cranial nerve nuclei. Six of the 7 animals showed involvement of one or more of the 3rd, 6th, 8th, or 10th (dorsal) cranial nerve nuclei. The earliest change was localized edema with separation of glial and nerve fibers and fragmentation and disintegration of myelin sheaths. A process resembling acute ischemic necrosis was seen in some instances. With progression of either type of lesion there was dis-

integration of axis cylinders, degeneration of glia and accumulation of many microglia and fewer astrocytes. The vascular proliferative reactions described in Wernicke's disease were occasionally seen but were not prominent. Structural changes were not demonstrable in the peripheral nerves and no significant lesions were seen in the spinal cord. These observations suggest that thiamine depletion might be a contributory or major factor in some cases of Parkinson's disease.

Blood Formation. Our experiments have shown the regular occurrence of a moderate anemia characterized by a suppression of reticulocytosis.

The Heart. Major interest has revolved about the effect of thiamine deficiency on the heart. Accumulated clinical and experimental evidence leaves little doubt that a functional and structural defect results. We have found the right side of the heart to be dilated, at times appearing as if the muscle were stretched. Histologic examination revealed small foci of myocardial necrosis as previously found in experimental thiamine deficiency in pigeons, rats, and pigs. Another lesion was a well defined hydropic degeneration of myocardial fibers with hyperplastic nuclear changes involving, particularly, subendocardial fibers, presumably of the conduction system. This lesion is like that described by Wenckebach in human deficiency.

Discussion

(Dr. Richard H. Follis, Jr., Baltimore, Md.) I should like to ask an obvious, but important question; that is, what supplements were given, and in what form were they given?

(Dr. John H. Fisher, London, Ont.) The cerebral lesions, which Dr. Rinehart described, are highly comparable to those seen in arsphenamine encephalopathy, particularly as to the character of the lesions, and their peculiar symmetrical distribution. I think it has been claimed recently that the lesions seen in arsphenamine encephalopathy are perhaps due to thiamine deficiency rather than to any actual direct toxic effect of the arsphenamine itself. I have observed a fatal case of arsphenamine intoxication in which, at autopsy, the brain showed multiple symmetrical foci of hemorrhagic necrosis, characteristic of arsphenamine encephalopathy. During the course of treatment the thiamine level in this patient's blood was greatly lowered.

(Dr. William Boyd, Toronto, Ont.) I should like to ask Dr. Rinehart if he has any comment to make on the possible relationship of these experimental lesions to those cases which Dock and his associates described of myocardial failure attributed to deficiency of thiamine or vitamin B complex. These cases were characterized by two pathologic changes. One was the remarkable increase in the weight of the heart; the average weight of the heart in the five or more cases was about 650 gm. The second point was a remarkable subendocardial fibrosis. I should be particularly interested to hear the distribution of the myocardial fibrosis in Dr. Rinehart's animals.

(Dr. Ralph D. Lillie, Washington, D.C.) Some 20 years ago when we were first separating B1 from the vitamin B complex, I had a series of some of the first rats in which a rather prolonged polyneuritis had been produced, and had the enjoyable experience of looking over their brain sections and found absolutely nothing to account for this. I am very much interested in Dr. Rinehart's success in demonstrating intracerebral lesions.

(Dr. Helen Ingleby, Philadelphia, Pa.) The myocardial changes shown are very similar to those of the human heart in beriberi.

(Dr. Alfred Angrist, Jamaica, N.Y.) In my experience, the myocardial changes noted occur quite commonly in routine human material, and we have always considered them as evidence of circulatory insufficiency (anoxic) of rather marked degree. I am reasonably certain that they will be found in the human being in cases in which no gross dietary deficiency can be demonstrated in the history.

(Dr. Rinehart) With regard to the question of Dr. Follis, the diet was essentially that used by Waisman, and is essentially a synthetic diet. The diet was fed to the animals in tablets.

In reply to Dr. Boyd's question, I think this does bear some resemblance to the condition of which Dr. Dock spoke. If it were a very prolonged experiment one might get subendocardial fibrosis of some degree. Most of the animals were subjected to two or more episodes of acute depletion. No doubt the pathologic changes were accentuated by this procedure.

In regard to Dr. Fisher's comment, I am much interested in his reference to arsphenamine. Possibly this inhibits the enzyme mechanism involving thiamine.

Thrombi were not found in the brain or in the heart muscle. Frankly I do not understand the pathogenesis of the lesions. I undertook the work in part to try to satisfy myself about the heart changes in thiamine deficiency. The literature is just a little confusing, and I thought with this experimenal background I would have a little better chance to recognize it in the human being. I would, however, hesitate to diagnose this cardiac lesion on histologic grounds alone. I would like to have confirmation of biochemical observations.

SYMPOSIUM ON HEPATIC INJURY

NECROTIZING HEPATIC INJURY AND ITS SEQUELS.* Balduin Lucké, Philadelphia, Pa.

LIVER NECROSIS PRODUCED WITH SODIUM TANNATE. F. W. Hartman, Detroit, Mich.

Abstract. Alkaline sodium tannate, pH 10, may be slowly injected intravenously with little or no immediate reaction on the part of the experimental animal. The solution of sodium tannate is made by dissolving 7.5 gm. of U.S.P. tannic acid in 100 cc. of distilled water and adding 10 per cent sodium hydroxide until a pH of 10 is obtained. Injection intravenously of 2 to 3 cc. of this solution per kg. in the dog or rabbit results usually in rapidly developing jaundice, loss of appetite, loss of weight, coma, and death. Autopsy shows all tissue icteric and the liver either large and congested, or small, flabby, and greenish yellow. The microscopic examination of the liver in most instances reveals a central necrosis of varying extent.

FATTY INFILTRATION, NECROSIS, AND SCARRING OF THE LIVER PRODUCED BY DIETARY MEANS AND MODIFIED BY THYROID ACTIVITY. Richard H. Follis, Jr.† and (by invitation) Philip Handler, Durham, N.C.

Abstract. The production of hepatic lesions by dietary means is now well recognized. When rats are placed on synthetic rations of low protein content with added cystine and containing varying amounts of fat, but no choline, extreme fatty infiltration of the liver is found. In time certain hepatic cells become necrotic and are replaced by scars. In some livers an acid-fast, fluorescent pigment, ceroid, appears; this is related to the cod-liver oil content of the diet. When other animals are placed on low protein diets without added cystine, but with supplements of choline, acute and widespread necrosis is found; such animals usually die rather suddenly. The lesions observed in choline or cystine deficiency are therefore different. Our observations have dealt with the modification of these lesions by thyroid activity, since we have noted differences in the hepatic lesions of animals whose choline-deficient diets contained sulfasuxidine. It will be recalled that

* By invitation of the Council.
† Present address, Baltimore, Md.

Gyorgy and Goldblatt have reported a protective effect when thiouracil is added to a choline-deficient regimen.

A. When anti-thyroid substances (sulfasuxidine, thiouracil, and para-amino-benzoic acid) are administered, the histological picture ordinarily produced by choline deficiency is changed. Such anti-thyroid substances lead to an accentuation of the fatty infiltration in the liver, yet seem to delay the production of necrosis and scarring; protection is by no means complete, however.

B. When thyroid extract is administered to choline-deficient animals, there is a reduction in the fatty infiltration. However, since such animals die relatively early, its effects on necrosis and scarring have not been determined as yet.

C. The effects of thyroid activity on cystine-deficient rats are equivocal. Added thyroid extract does not seem to retard or accelerate necrosis in any degree. We have not studied the effects of anti-thyroid substances on a cystine-deficient regimen.

D. As is well known, sulfasuxidine, para-amino-benzoic acid, and the thiouracils lead to anatomic thyroid hyperplasia but physiologic thyroid hypofunction. Thyroid extract leads to anatomic thyroid hypoplasia and physiologic glandular hypofunction. It is of some interest, therefore, that choline deficiency leads to anatomic thyroid hyperplasia. Whether there is an accompanying hyperfunction, eufunction, or hypofunction remains to be determined. It is of interest, however, that this observation is compatible with morphologic and chemical data available on the relationships between thyroid function and liver fat.

E. Raising the fat content of the diet increases the fat in the liver of choline-deficient rats and tends to accentuate the necrosis of cystine deficiency.

UNANTICIPATED LIVER CHANGES IN DOGS FOLLOWING CESSATION OF A HIGH-FAT DIET. Russell L. Holman, New Orleans, La.

Abstract. During experiments which involved feeding dogs a specified high-fat diet for 2 months or longer, followed by an interval of 1 to 4 weeks of kennel diet without fat supplement, then sacrifice with induced renal insufficiency (uranium nitrate), marked increases in liver weight associated with occasional histologic changes in the liver were noted. When these experiments were controlled by using a similar period of high-fat feeding, followed by similar intervals of kennel diet without fat supplement, but instead of heavy metal injury the dogs were sacrificed with a blow on the head, the increases in liver weight did not occur, but the histologic changes were more marked. All 4 dogs subjected to this latter procedure showed atypical focal necrosis in the liver and in one of these there were, in addition, early cirrhotic changes. In a large number of control dogs no such changes have been observed. These unanticipated findings, which obviously need confirmation and extension, suggest that simple withdrawal from a high-fat diet may not be without some dangers. They further suggest that fat absorbed from the gastro-intestinal tract may pass through the liver with greater ease than fat resorbed from the depots.

HEPATIC NECROSIS INCIDENT TO SHOCK. Virgil H. Moon, Philadelphia, Pa.

Abstract. Hepatic degeneration and necrosis occur in secondary shock resulting from wounds, burns, intoxications, infections, poisons, heat stroke, and from other causes. Corresponding changes are found after experimental shock produced by similar agents. The necroses vary in amount and in location. Scattered groups of necrotic cells occur in central, mid-zonal, or in peripheral areas of the lobules; often necrosis is most marked in the central area. In exceptional cases, as after extensive burns, heat strokes, or severe intoxication, the liver resembles "acute yellow atrophy." The cause of these hepatic effects is obscure. Toxic substances from burned or infected areas have been suggested. However, the same effects

are seen after shock from lack of oxygen, heat stroke, anaphylaxis, and from low barometric pressure as in high-altitude aviation, in which instance no source for toxic substances is apparent. Anoxia is a common denominator in shock from diverse causes; perhaps degeneration and necrosis result from anoxia.

A CYTOCHEMICAL STUDY OF REGENERATING RAT LIVER. Robert E. Stowell, St. Louis, Mo.

Abstract. Following the removal of 60 per cent of the liver of adult rats by partial hepatectomy, the regenerating liver was studied at frequent time intervals up to 2 weeks. Sections of surgically removed (control) tissue and of regenerating liver which were frozen-dried by the Altman-Gersh method or fixed in Stieve or Carnoy fluid were (1) photographed in ultraviolet light of 2750 Å, or (2) stained with hematoxylin and eosin, or (3) with the Feulgen reaction for thymonucleic acid. Six hours after partial hepatectomy, vacuolization of the cytoplasm of the hepatic cells was evident and the cells continued to increase in volume up to the time of increased mitotic division. The mean size of the nucleoli, which by 24 hours had increased 3½ times, returned to normal by 48 hours. The number of nucleoli per nuclear section increased during the second day. The increased light absorption in regenerating liver at 2570 Å, which is characteristic of nucleic acid, was confirmed by macrochemical analysis showing a significant increase in total phosphorus and in percentage of nucleotides of the ribose type. The results illustrate the relationship of the nucleolus, ribose nucleic acid, and protein metabolism of hepatic cells.

Discussion

(Dr. Alfred Angrist, Jamaica, N.Y.) This work of Dr. Stowell has been of considerable interest to us, and we find it has a distinct application to the subject of our symposium.

We have been studying nucleic acid partitions in regenerating liver. Our work was initiated by finding that in ordinary old and advanced Laënnec's cirrhosis, in which the morphologic pathologist feels that the liver tissue is quite static, we got figures by chemical analysis such as obtain for a more youthful type of cell than the normal, approaching data we get in embryonic tissue or in a tissue that is rapidly regenerating. The proportion of ribose nucleic acid to desoxynucleic acid was determined by using the fractionating method of getting different proportions extractable at different temperatures, which is the technic used in ordinary polymer research (and I think that everybody is agreed that nucleic acid exists in the body in polymeric form). I repeat that our results, suggesting a shorter polymeric chain structure of the nucleic acid moiety, hold true for ordinary cirrhosis.

We carried the work further to attempt observations on "butter yellow" livers, in which obvious regeneration occurs. When we tried to use the large tumorous nodules for chemical analysis, with the remaining nontumor-bearing liver of such animals as a comparison for control, we found that the apparently normal liver in "butter yellow" animals was unsuited as normal control material because it showed intermediate values between those for the true hepatomas or cholangiomas of the "butter-yellow" livers and those obtained from livers of rats on normal diets without the "butter-yellow." This indicated a regenerative process not obvious morphologically, in keeping with Dr. Stowell's findings without any butter-yellow.

The significance of these findings, I think, is to be found in the insight it gives us into the biochemical significance of the nucleoproteins in relation to cellular regeneration. Our initial work, which will be reported later this week before the Society for Experimental Biology and Medicine, seems to indicate that it is not only the total quantity of the nucleic acid, which Dr. Stowell has presented, but also the state of the nucleic acids, as far as their chain formations, in long or

short chains of varying length for each of the molecules, which is important. This may be a little obtuse at the moment, but it surely bears further study.

EPIDEMIC HEPATITIS WITH RUPTURE OF THE SPLEEN IN THE PRE-ICTERIC PHASE. A REPORT OF A CASE. John L. Work and (by invitation) H. Garrett Van der Veer, Montclair, N.J.

Abstract. A previously healthy 27-year-old white man was operated upon 4 days after the onset of chills, fever, nausea, vomiting, and abdominal cramps. The laparotomy revealed hemoperitoneum due to rupture of the spleen, and a splenectomy was performed. Jaundice developed on the third postoperative day, and the patient died 5 days later. The spleen was slightly enlarged and microscopic sections revealed severe hyperplasia of the pulp. The most important abnormalities disclosed by the autopsy were in the liver. The changes were those which have been described as characteristic of epidemic hepatitis. There was also right-sided dilatation of the heart, bilateral bronchopneumonia, edema of the esophagus, stomach, and intestines, acute enteritis and colitis, and ascites.

SEQUEL TO NECROTIZING HEPATITIS. Max M. Strumia, Bryn Mawr, Pa.

Abstract. A 43-year-old male was first admitted to the hospital in September, 1945, because of compression fractures of the 4th and 5th dorsal vertebrae, fractured clavicle, and cerebral concussion following an automobile accident. The patient's history revealed jaundice 20 years prior to admission, lasting 2 weeks. The patient remained in a body cast for 3 months, during which time he ate poorly and lost considerable weight. He did not receive blood, plasma, or serum. In April, 1946, slight jaundice was noted, which became progressively worse. He was readmitted on May 30, 1946, with pitting edema of the ankles, enlarged liver, and palpable spleen. Laboratory studies showed a serum bilirubin of 14 mg.; cholesterol, 113 mg.; cholesterol esters, 26 per cent; phosphatase, 6.2 Bodansky units; total protein, 4.6 gm. per 100 cc.; albumin, 2.9 per cent; prothrombin, 45 per cent of normal; urea nitrogen, 5 mg. per 100 cc.; nonprotein nitrogen, 44 mg. per 100 cc.; and markedly increased bromsulfalein retention. He was treated with high-protein, low-fat, high-carbohydrate diet and with protein feedings intravenously. Peritoneoscopy revealed a cirrhotic liver. Four thousand cc. of ascitic fluid were removed. The patient improved considerably and was discharged on June 14, 1946. He did fairly well at home until the morning of June 24, when he had a sudden attack of coughing, followed by vomiting bright red blood. He rapidly became unconscious and died shortly after. The autopsy revealed remnants of a necrotizing lesion typical of infectious hepatitis, a typical post-necrotic cirrhosis. It is felt that without the esophageal hemorrhage this patient might have survived with development of a full-fledged cirrhotic lesion.

CIRRHOSIS FOLLOWING INFECTIOUS HEPATITIS. Walter H. Sheldon and (by invitation) David F. James, Atlanta, Ga.

Abstract. Infectious hepatitis, after the subsidence of acute symptoms, often leaves impaired liver function. This has been substantiated by many clinical but relatively few morphologic observations. The latter have created confusion as to the type of lesion resulting, which has been classified as Laënnec's cirrhosis, "nodular" cirrhosis, or as "cholangiolitic" cirrhosis. We have studied 3 cases in which we believe infectious hepatitis ended in a lesion described by F. B. Mallory as "toxic" cirrhosis. Two of our patients had bouts of hepatitis 5 years before their final admission. Interim studies available in one showed persistence of impaired hepatic function. The clinical picture on final admission of all 3 patients was that of hepatic failure with portal hypertension.

The autopsy findings in the 3 patients were similar. The livers were small and

irregularly nodular. The tissue between the nodules was shrunken and atrophic. The cut surfaces showed complete loss of normal architecture. On histologic examination the nodules consisted of regenerated liver cells separated by wide areas of stroma which was collapsed and devoid of parenchyma. The liver cells in many nodules displayed slowly progressive degenerative changes of varying, often considerable, extent, ranging from fatty metamorphosis to necrosis. The central and midzonal portions were chiefly involved, while the peripheral cells showed regeneration. The stroma in the areas devoid of liver cells showed preservation of the lobular pattern, without new formation of either reticulum or collagen. Similarly, the portal spaces revealed only condensation of the stroma without scarring. A mild chronic inflammatory infiltration was present. Bile casts were frequent in the intercellular bile canaliculi. The perilobular bile ducts were increased in number, while the larger ducts were not remarkable. Other organs showed the effects of portal hypertension, with splenomegaly and esophageal varices.

The active degenerative changes indicated the progressive character of the lesion, which otherwise was similar to subacute infectious hepatitis. These changes may be secondary to ischemia and accumulated metabolic products, but one might speculate that persistence of the viral infection could account for this picture. Our findings are identical with the lesion described variously as "toxic" or "post-necrotic" cirrhosis and as "healed acute yellow atrophy." We conclude that infectious hepatitis sometimes results in chronic progressive liver disease identical with "toxic" or "post-necrotic" cirrhosis. The lesion can be distinguished from other types of cirrhosis.

INFECTIOUS VS. TOXIC HEPATITIS IN THE CIVILIAN POPULATION. Hans Popper and (by invitation) Murray Franklin, Chicago, Ill.

Abstract. This paper is based on a study of all cases of fatal hepatitis observed at Cook County Hospital in the past 18 years. The differences are discussed which exist between the infectious (viral) form, which is similar to the great majority of the cases observed in military personnel, and the different forms of toxic hepatitis which represent the majority of the cases in our material. The criteria available in post-mortem material have been applied to a series of liver specimens obtained for biopsy by needle from cases of primary hepatitis. As a result, in the majority of these patients a differentiation into toxic and infectious groups was possible.

XANTHOMATOUS BILIARY CIRRHOSIS.* H. Edward MacMahon, Boston, Mass.

Abstract. A histologic study of adequate "surgical biopsies" from the livers of 4 patients considered by Thannhauser as classical examples of a syndrome which he called "xanthomatous biliary cirrhosis" revealed a chronic inflammatory reaction throughout the portal areas. This reaction was most concentrated about the terminal and junctional bile ducts. There was a proliferation of inflammatory granulation tissue, a moderate cellular infiltration, a disappearance of terminal bile ducts, degeneration and necrosis of liver cells at the periphery of the lobules, regeneration of liver cells with formation of new junctional ducts, and, finally, moderately severe intralobular bile stasis. At times the inflammatory reaction cut deeply into the lobules and isolated islands of liver cells. For the most part, the lobular pattern and the central veins, central zones and mid-zones were well preserved. No xanthoma cells were found in any of the sections. No bile or leukocytes were seen in any of the bile ducts. No organisms were demonstrable. The picture was one of chronic pericholangiolitis with early and uniform perilobular fibrosis. It had features in common with both obstructive and cholangiolitic cirrhosis, but it was distinct from each of them.

* This article will appear in a subsequent issue of *The American Journal of Pathology*.

Because the anticipated lesion of xanthomatous biliary cirrhosis of the liver could not be substantiated in sections, it is suggested that this name as applied to cirrhosis should be discontinued. To indicate the original site of the inflammatory reaction, to denote the inflammatory nature of the lesion, and to indicate the ultimate fate of the liver, the term "pericholangiolitic biliary cirrhosis" is suggested to indicate the type of disease found in the four cases examined.

Discussion

(Dr. Paul Kimmelstiel, Charlotte, N.C.) In view of the rarity of this condition, it may be of interest to mention a case of so-called xanthomatous cirrhosis which we observed several years ago. The case was that of a middle-aged woman with widespread xanthomatosis of the skin, extremely high cholesterol level of the blood, and gradually increasing jaundice and ascites, fulfilling all of Thannhauser's criteria. The post-mortem examination showed an ordinary Laënnec's cirrhosis of the liver. No xanthomatous lesions were found in extrahepatic or intrahepatic bile ducts and the histologic sections did not suggest biliary cirrhosis. I am inclined to agree with Dr. MacMahon that it is questionable whether the concept of xanthomatous cirrhosis as a disease entity is worth being maintained.

(Dr. Howard T. Karsner, Cleveland, Ohio) There are two questions I would like to put to Dr. MacMahon. The first has to do with the designation. As I have seen the lantern slides, I wonder if at the beginning this is not what Rössle called cholangitic, and subsequently what may have become, and probably did become, cholangiolitic. The other question is this: In talking with several of my clinical colleagues about cases in which they have made the diagnosis of xanthomatous cirrhosis, I have found that in each instance (the number is very small, 3 or 4), jaundice preceded the development of xanthomatosis by several months. That raises the question, then, as to whether the relationship is xanthomatosis-cirrhosis, or cirrhosis-xanthomatosis.

(Dr. MacMahon) In answer to Dr. Karsner's question in regard to whether this is cholangitic cirrhosis, as the term was employed by Professor Rössle: It was my good fortune to have spent a number of years with the late Professor Mallory, and to have familiarized myself with the lesion which he described as "infectious cirrhosis." This was an infection of the small bile ducts and was considered by him to be bacterial in origin. I also had the opportunity to work on the liver under Professor Rössle in Berlin. At that time I showed him slides which Dr. Mallory had called "infectious cirrhosis" and these he called cholangitic cirrhosis. The lesion in the liver which I have presented today and called "pericholangiolitic biliary cirrhosis" simulates the cholangitic cirrhosis of Rössle (infectious cirrhosis of Mallory) but may be distinguished from it. The characteristic features of cholangitic cirrhosis (Rössle) are an inflammatory exudate within the lumina of the interlobular bile ducts, a proliferation of these ducts, and an unevenness in the extent and distribution of the inflammatory lesion. In pericholangiolitic biliary cirrhosis during an equally active phase, there are no leukocytes in the bile ducts, the inflammatory reaction is concentrated about the cholangioles, there is a diminution or complete loss of small bile ducts, and the lesion is strikingly uniform in distribution. Under low magnification, these two diseases have much in common, but I believe it is possible, particularly in the early phase, to distinguish one from the other. At this point, it must be admitted that the lesions in pericholangiolitic biliary cirrhosis resemble more closely a type of biliary cirrhosis described by Rössle under the name of "cholangiolotische Zirrhose" (cholangiotoxische Zirrhose). Because no toxin has been definitely identified in association with the lesions which I have just described, the purely descriptive term of "pericholangiolitic biliary cirrhosis" has been chosen.

In these 4 cases and in all cases that have been reported in the literature as show-

ing this clinical syndrome, jaundice has preceded xanthomatosis by at least 6 months, and in some cases by as long as 2 years. It was an interesting finding that of all the cases recorded, 87 per cent have been in females, irrespective of the underlying changes in the liver. The average age was about 40, and it is perhaps worth mentioning that for many months after the appearance of jaundice the great majority of these patients have remained remarkably well.

POSSIBLE RELATION OF DUODENITIS AND DUODENAL ULCER TO HEPATIC CIRRHOSIS.
William Carpenter MacCarty, Sr., Rochester, Minn.

Abstract. In the symposium of twelve papers of which this is a part, I am glad to see that the term cirrhosis has been used only three times in the titles. Fortunately, and perhaps correctly, we are beginning to speak of chronic hepatitis, an expression which includes the late stages described long before we had opportunities to study the early stages at surgical exploration. Among the many causes of advanced chronic hepatitis, one rarely hears of duodenitis and duodenal ulcers and their possible relation to this condition. As long ago as 1872, 1876, 1882, and 1908, Meyer, Charcot and Gombault, Maffucci and Tsunoda described, respectively, the experimental production of biliary cirrhosis by artificial stenosis and partial stenosis of the common duct, which is not an uncommon surgical condition produced by duodenal ulcer. Formerly it was thought that most duodenal ulcers were single and near the pylorus; duodenal ulcers are, however, very often multiple and exist just above, at, and just below the papilla of Vater. In these portions of the duodenum it is quite reasonable to think of repeated interference with the patency of both the common bile and pancreatic ducts, thereby producing unfavorable changes in both the liver and the pancreas. From a large series I have chosen two examples of the association of cirrhosis with duodenal ulcers which partially obstructed the common duct.

Case 1. A male, aged 60 years, was operated upon for cholecystitis 17 months before the last operation. He improved for 5 or 6 months and again had epigastric pains, jaundice, and vomiting. At the second operation the gallbladder was distended, the common duct dilated, and the head of the pancreas very hard. The patient died on the 12th day following the re-operation and the necropsy showed a marked chronic hepatitis (called cirrhosis), chronic cholecystitis, chronic pancreatitis, chronic congestion of the spleen, and a chronic ulcer of the duodenum at the papilla of Vater about 1 cm. in diameter. There was almost complete stenosis of the common duct at the base of the ulcer.

Case 2. A male, aged 58, 4 years before examination had had attacks of severe epigastric pain which radiated to the back. Seven years before the last operation he had similar attacks. At operation one gallstone was removed and the gallbladder was drained. There was a hard mass felt at the end of the common duct, thought clinically to be a carcinoma. The patient died on the 4th postoperative day from a hemorrhage into the bowel. At post-mortem examination there was a duodenal ulcer 2 cm. in diameter with the common duct running through the base of the ulcer. There was a very definite biliary hepatitis, called cirrhosis. The surgeon noted that the liver was "angiomatous."

My purpose is merely to stimulate others to watch for this association at autopsy and at surgical exploration. Even experimental production of partial or periodic occlusion of the common duct and pancreatic duct might throw light on some of the diseases of the liver and pancreas which have been considered primary entities rather than sequelae of duodenal pathologic conditions.

Discussion

(Dr. Max M. Strumia, Bryn Mawr, Pa., replying to a comment by Dr. MacCarty) In my abstract of the case which I reported today, I stated that the

stomach contained 400 cc. of clotted blood; the small intestine was filled with tarry material, presumably blood. The mucosa of the stomach was flattened. There was a break in one esophageal vein which measured 3 mm. The mucosa of the duodenum was thickened and red. Most of the mucosa of the cecum was pale and showed no gross lesions. The remainder of the gastrointestinal tract appeared normal. Histologic examination showed that the duodenum was congested. The colon seemed to show a mild chronic colitis.

INJURIES PRODUCED BY THE ATOMIC BOMB
OBSERVATIONS ON HUMAN BEINGS AT HIROSHIMA AND NAGASAKI

MECHANICAL INJURIES AND BURNS. Averill A. Liebow and Shields Warren, New Haven, Conn., and Boston, Mass.

Abstract. Direct blast injury analogous to that inflicted by high explosives was almost unknown among survivors at Hiroshima and Nagasaki as indicated by an incidence of ruptured eardrums of about 1 per cent. Almost universal, however, were injuries produced by flying glass and the falling beams of wooden houses. The more severe injuries were rare since those that had been severely injured were killed by fires that swept the city before rescue operations could be instituted. The burns among survivors were largely of the "flash" type, the result of an exceedingly large quantity of radiant heat acting for an exceedingly brief interval. Only survivors in the rectilinear path of the rays were involved, so that the burns were of a sharply outlined "profile" or "mask" type. Depigmentation at the center with marginal hyperpigmentation of the burns was prominent in patients close to the bomb, but at greater distances the entire exposed surface became intensely pigmented, and the pigment showed no tendency to fade within 4 months. There was histologic evidence that the depigmentation occurred without destruction of the squamous epithelium of the surface, suggesting the action of specific wave lengths. Even minor injuries and burns became serious foci of infection in persons who also suffered the leukopenia resulting from radiation.

EARLY EFFECTS OF RADIATION. Averill A. Liebow and Shields Warren, New Haven, Conn., and Boston, Mass.

Abstract. The effects of the ionizing radiations resembled closely those produced by x-rays in animals and men. Nausea and vomiting occurred in many adequately exposed persons within a few hours after the bomb. A special effort was made to center the study of the radiation effects upon tissues from patients who had sustained little or no other injury. The earliest autopsy material is from persons dying "mysteriously" with symptoms of severe diarrhea and fever on the third day after the bomb. In them, epilation and the clinical manifestations of aplastic anemia (except leukopenia) had not had time to appear.

The Skin. In a few cases there were epithelial changes at the margins of ulcerative lesions in patients dying in the third week, but since most persons who received as much as an erythema dose over the whole body died during the first confused days when autopsies were extremely rare, little material is available for study. Epilation in both men and women began 14 to 20 days after the bomb. It involved chiefly the scalp in a distribution resembling that of ordinary baldness. Histologically, the mechanism is entirely analogous to that of the usual processes of loss and replacement of the hair, with arrest of mitosis in the matrix, failure of differentiation of the internal root sheath as the old hair is extruded, and finally (some 2 months after the bombing) evidences of renewed differentiation of the internal root sheath with penetration of the new hair through the old external sheath to the surface.

Gastrointestinal Tract. Typical radiation changes were seen in the intestines of

persons dying as early as the fourth day. These consisted of bizarre cells, some with enormous nuclei possessing a coarse chromatin network and a large body of cytoplasm. Atypical mitotic figures were found in some cells and tripolar mitotic figures were observed. In one patient dying on the tenth day, the cytoplasm and nuclei of the squamous epithelial cells were remarkably swollen, and fragmentation of the nuclei was observed.

Gonads. Even at the fourth day remarkable changes were found in the testes, with sloughing of the germinal epithelium, together with an increase in Sertoli cells. Toward the end of the first month, there was almost complete loss of germinal epithelium. After the fifth week the tubules began to display thickening of the basement membrane and there were hyaline deposits restricting the lumina of the interstitial blood vessels. There was slight hyperplasia of the interstitial tissue after the end of the sixth week. To correlate with the changes in the testes, there was a remarkable decrease in the count of the spermatozoa of patients who had been close to the bomb. How permanent this will be is at the present time unknown. Occasionally, "castration cells" were found in the pituitary glands.

Much less striking changes were observed in the ovary. A few primary follicles were in process of atresia. The most usual finding was that of the absence of developing follicles despite the persistence of primary follicles. The endometrium showed an absence of corpus luteum effect.

Lymphoid Tissues. Even after 3 days there was a remarkable degree of atrophy of the lymphoid tissues, leaving nothing but the reticular skeleton. Beginning on the fifth day, however, large numbers of atypical mononuclear cells resembling lymphoblasts or Reed-Sternberg cells began to appear. These gradually decreased in number during the following 3 months and in a few instances secondary follicles had reappeared by this time both in the spleen and lymph nodes.

Bone Marrow. Even within the first 4 days there was, in severely exposed individuals, almost total loss of all myeloid and erythroid tissue, but there was evidence of proliferative activity, with new reticulum cells, often as bizarre as those seen in the lymph nodes, making their appearance. During the first month such proliferative activity became remarkable in many cases, but the products were largely atypical reticulum cells and plasma cells. In some patients, at variable intervals, there was renewed differentiation into granulopoietic and erythropoietic tissue, and in some, who died toward the end of the sixth week, there was actually hyperplasia of these cells, although peripheral leukopenia had been noted.

PATHOLOGIC ANATOMY OF LETHAL IONIZING RADIATION: LATE DEVELOPMENTS. Elbert DeCoursey, Fort Sam Houston, Tex.

Abstract. The prominent lesions are presented which are seen in human beings dying from 7 to 15 weeks after instantaneous ionizing radiation from an atomic explosion. Not as many individuals of this group had had nausea and vomiting on the first day or as severe leukopenia, purpura, or oropharyngeal lesions as had those dying earlier. Emaciation is the usual finding. The scalp, femoral bone marrow, spleen, and intestines have striking gross changes, and the bone marrow, lymph nodes, and testes have distinctive microscopic changes.

In the skin, hemorrhagic lesions become uncommon, hair follicles begin to regenerate, decubital ulcers are usually present, and most of the flash burns are healed, but some, still granulating, appear keloidal.

The femoral bone marrow is usually pink in the upper third or half and all marrow shows more and more myeloid hyperplasia but usually with some maturation defect. Severe anemia has usually been present before death.

The spleen and lymph nodes remain atrophic.

The testes are more atrophic and contain almost no spermatogenic cells, the Leydig cells being apparently uninvolved.

The adrenals are uniformly small and lipoid-deficient.

The gastrointestinal tract presents the most striking changes, which vary from petechiae to widespread pseudomembranous ulcerative involvement resembling bacillary dysentery. These lesions and necrotizing pneumonia are the leading causes of death. The heart, thyroid, salivary glands, brain, liver, pancreas, prostate, kidneys, and bladder show no constant radiation-connected changes except for occasional petechiae or a rare bacterial clump. There is no effect on growing cartilage or bone.

PATHOLOGY OF THE EYE IN ATOMIC BOMB CASUALTIES. Helenor Campbell Wilder (by invitation), Washington, D.C.

Abstract. The following observations were based on the microscopic examination of 18 eyes removed at autopsy from Japanese who died 24 to 33 days after the Hiroshima bombing. In no instance was an entire globe or both eyes of a patient received.

Breaks in Bowman's membrane were seen in 4 eyes and may have been the result of direct injury. They also occur as senile phenomena. In 6 eyes there was elastosis of the conjunctival stroma. This, however, is an almost constant finding in persons over 50 years of age and may occur in young people as a result of exposure.

In one instance there was hemorrhage in the conjunctiva; in another, a small pre-retinal hemorrhage with a few red cells streaming out into the vitreous chamber; and in a third, choroidal hemorrhage. In one retina there were small, apparently edematous areas in the nerve fiber layer. In these the nerve fibers appeared swollen, and occasional homogeneous structures were somewhat suggestive of the cytoid bodies one sees in hypertensive vascular disease with renal retinopathy, and in choked disk. Although this patient was only 23 years old, the lesions must be considered as possibly related to central serous chorioretinitis which is common in Japanese, but affecting, particularly, men of middle age. They probably correspond to the white spots in the fundus observed by Flick.

Septic choroiditis was present in 17 of the 18 eyes. It was manifested by infiltration, particularly of the posterior choroid, by large mononuclear cells occasionally in mitosis, and a scattering of lymphocytes, plasma cells, and Russell bodies. Large mononuclear cells frequently packed choroidal veins, but were rarely found in the arteries. In one case bacilli resembling those found in the bone marrow of the same patient engorged many of the capillaries of the choriocapillaris.

Degenerative changes were seen in the lens in 15 eyes; in 2 eyes the lens was not included in the blocks, and in one it appeared normal. The changes were limited to the cortical fibers and were more prominent at the posterior pole. Here the fibers were swollen and pale-staining. Vacuoles of various sizes appeared immediately beneath the capsule, which was wrinkled over the larger ones. Anteriorly and at the equator the changes were usually less marked, the vacuoles smaller, and the overlying subcapsular epithelium intact.

Of the pathologic changes in these eyes, cataract alone can be regarded with any degree of plausibility as the direct effect of irradiation. Blood dyscrasia, in itself an effect of irradiation, was the probable cause of the hemorrhages and a possible cause of the retinal edema; septic choroiditis was associated with the consequent severe infections accompanied by septicemia. Breaks in Bowman's membrane and elastosis of the conjunctiva were of doubtful origin and may even have been unrelated to the explosion.

HEMATOLOGY. George V. LeRoy (by invitation), Chicago, Ill.

Abstract. The disturbances in the blood and the blood-forming organs of the Japanese exposed to the atomic bomb were of a type that could be anticipated on

the basis of experimental studies of animals exposed to large single doses of ionizing radiations. The most important observations that were made in Japan concerned the rate at whch aplastic anemia developed, and the character and tempo of the recovery process, when it occurred. In the most heavily irradiated individuals the syndrome was fully developed and death occurred within a period of approximately 2 weeks. Even in such patients, however, there was evidence of some proliferation of the reticulo-endothelium of the hemopoietic system. In less heavily irradiated individuals the maximum depression of the activity of the blood-forming organs occurred between the 4th and the 6th week after exposure. The leukocyte count, as an average, was lowest during the 4th and 5th week, and had returned to normal levels between the 8th and 9th week after the bombing. The erythrocyte count and the thrombocyte count reached the lowest levels during the 6th to the 8th week, and returned to normal values at about the 12th to the 16th week after the bombing. The tendency of the bone marrow to regenerate was observable in all specimens studied. In some patients it appeared that regeneration proceeded from normal hemopoietic elements that had survived irradiation. In others, proliferation of the reticulo-endothelium with the evolution of functional marrow from the products of this system was observed. In some preparations there was evidence of the development of neutrophilic myelocytes directly from the reticulo-endothelium without the appearance of recognizable myeloblast-like forms. The clinical significance of the tendency for heavily irradiated bone marrow with aplastic anemia to recover is of obvious importance in the management of irradiated patients.

OBSERVATIONS ON ANIMALS EXPOSED AT BIKINI

MECHANICAL INJURIES AND BURNS IN THE BIKINI ANIMALS. R. Harold Draeger (by invitation), Washington, D.C.

Abstract. In Operation Crossroads, goats, pigs, and rats were exposed on target ships at Bikini during Tests Able and Baker in order to determine the probable effects of an atomic bomb explosion upon naval personnel. About 30 per cent of the animals died from the effects of the bombs. The majority of these deaths were caused by ionizing radiation; about 10 per cent of the fatalities were due to air blast. Flashburn was not an important factor since the fur of the animals in most instances provided efficient protection.

THE PATHOLOGIC CHANGES INDUCED BY IONIZING RADIATIONS IN THE BIKINI ANIMALS. John L. Tullis (by invitation), Washington, D.C.

Abstract. The animals which received a fatal dose of ionizing radiation during the atomic bomb tests at Bikini died within 1 month after exposure. There have been no deaths due to latent or chronic radiation injury. The gross pathologic lesions were, in general, of three types: (1) widespread hemorrhage throughout the organs of the body, causing severe anemia; (2) secondary infections, usually involving only the lungs; (3) degenerative changes, manifest chiefly as ulcerations of the gastrointestinal tract and tonsillar tissue.

THE HEMATOLOGY OF THE BIKINI ANIMALS. Eugene P. Cronkite (by invitation), Washington, D.C.

Abstract. In Bikini Test Baker, animals were exposed to massive fatal doses of ionizing radiations over a period of 4 to 5 days. These were of two magnitudes: the greater dose resulted in a marked leukopenia involving all cells and slight hemorrhagic phenomena at a time when platelets were present and the clotting time was normal; the lesser dose resulted also in similar changes in the leukocytes and in a severe hemorrhagic diathesis that may be divided into three stages. In the early stage there was increased capillary fragility with scattered petechiae. In the

second stage there was severe thrombocytopenia accompanied by purpura, while in the terminal stage the clotting time was prolonged. These changes resulted in an extensive purpura with both subcutaneous and subfascial hemorrhages. In the terminal stage the clinical and laboratory findings resembled those in purpura haemorrhagica and hemophilia.

Discussion of Papers on Injuries Produced by the Atomic Bomb

(Dr. Virgil H. Cornell, Washington, D.C.) I would like to ask if any experiments have been conducted with irradiated foods in otherwise unexposed animals, and, if so, whether gastrointestinal lesions were seen in such animals.

(Dr. Sol Roy Rosenthal, Chicago, Ill.) The marked tissue destruction following radiation and thermal exposure must indubitably have released into the blood stream protein split products, amongst these peptone, histamine, adenylic acid, and enzymes such as trypsin. The hemorrhage and shock following the injections of any of the substances mentioned are well known. I would like to know whether studies were made of the blood, either chemically or by injection into animals, to determine if such toxic products were actually released and whether or not it had been considered that these products might have attributed, in large measure, to the pathologic picture.

(Dr. Max M. Strumia, Bryn Mawr, Pa.) I would like to ask Dr. DeCoursey if any relationship was noted between the occurrence of intestinal lesions and the neutropenia, in view of the fact that in human patients suffering from malignant neutropenia or agranulocytosis, identical lesions are a common occurrence.

(Dr. Jacob Furth, New York, N.Y.) I would like to ask Dr. DeCoursey about the late effect of atomic bomb rays on the ovary, the organ in which neoplasms are most likely to occur after gamma irradiation. I also would suggest or question a correlation between the increased number of mast cells in tissues, as presented in the first paper, and the disturbance in blood clotting mechanism presented in the last.

(Dr. Austin M. Brues, Chicago, Ill.) Is it possible for Dr. Cronkite to give a breakdown of the 1300 and 1500 r. mentioned in his paper? Was that mostly at the time of blast from "gamma radiation," or was it partly from the beta- and gamma-emitting fission products dispersed at the time of the explosion and acting over a longer period of time?

(Dr. H. Edward MacMahon, Boston, Mass.) In view of what we have just seen and heard, it seems pertinent at this time to offer a word of warning about the indiscriminate use of radioactive substances in civilain life. It is one thing to use a radioactive substance for therapy in malignancy; it is quite another thing to use such a substance as a diagnostic aid. I have recently done an autopsy on a patient who received thorotrast,* which is radioactive thorium dioxide. This was given as a diagnostic aid. The autopsy showed not only very severe changes throughout the hematopoietic system similar to those that have just been described, but also a rapidly growing primary malignant endothelial cell sarcoma. This tumor arose in the liver in the immediate site of the greatest concentration of radioactive thorotrast in the body.

(Dr. Alfred Angrist, Jamaica, N.Y.) I would like to ask Dr. Wilder concerning the degree of change noted in these people with cataracts, and whether the patients who recovered showed changes in the lens, i.e., whether the changes were sufficient to be recognized as subcapsular cataracts by the usual clinical means of detection.

(Dr. Liebow) In regard to the question about the mast cells, it was observed that these were numerous, not only in the lymph nodes, but also in the intestines of persons dying approximately 1 month after the bomb. They became a very prominent feature of the cellular content. This is of interest in relation to the observa-

* *Am. J. Path.*, 1947, 23, 585-611.

tion made by Dr. Jean Oliver of Brooklyn on mast cell tumors in dogs, from which a large quantity of heparin can be extracted. Among these animals there was no evidence of change in the coagulability of the blood, but we do not know the significance of this.

(Dr. DeCoursey) In regard to the question concerning the relationship between neutropenia and intestinal inflammation—yes, there usually was a direct relationship in that the patient had a history of previous leukopenia, usually long-continued. Three weeks after the Nagasaki explosion, Japanese hematologists reported zero white blood cells in about 26 per cent of a group of people who had been within 2,000 meters of the bomb.

In answer to Dr. Furth, the ovary shows surprisingly little effect: The primary follicles are numerous. The most constant finding is an absence of developing follicles. Hemorrhages are the only other feature.

A real difference between air and under-water detonation of an atomic bomb is that the radiations from an air explosion are instantaneous; prolonged ionizing radiations are emitted from the materials in the falling water resulting from underwater detonation.

(Dr. Wilder) I am sorry I cannot answer Dr. Angrist's question concerning the clinical manifestations of cataract and the ultimate results in patients who recovered, as we had no slit-lamp examinations on these patients, and the eyes examined at the Institute were all removed at autopsy.

(Dr. LeRoy) In answer to Dr. Rosenthal's question concerning the concentration of nonprotein nitrogen in the blood of the Japanese victims of the atomic bomb, some of the research groups studied this factor 3 to 4 weeks after the bombing, and found no significant variations. I am not aware of any estimations of the trypsin content of the blood. Such studies as were made of other secretions of the gastrointestinal tract were normal.

When I returned to Chicago after studying the atomic bomb material I discussed the matter of the hemorrhagic state with Dr. J. Garrott Allen whose demonstration of the presence of heparin-like material in the blood of experimentally irradiated animals was of great interest to me. I told him that our pathologic specimens contained unusual numbers of mast cells, an observation of considerable significance in view of the relationship between mast cells and heparin production. I was pleased to find that Dr. Cronkite was able to corroborate some of Allen's findings, since his observations are very significant. The demonstration that an anticoagulant substance occurs in irradiated subjects, and that this material can be neutralized by appropriate means, is very important from the clinical standpoint. There are many difficulties involved in the use of toluidin blue for this purpose, and investigations are being conducted in our own laboratory to find other, and more reliable means of accomplishing this.

(Dr. Tullis) In reply to Colonel Cornell's question, all types of food were exposed to ionizing radiations at Bikini. It was not fed to the animals because the food did not become radioactive.

(Dr. Cronkite) In regard to the question about histamine, peptone, and trypsin, we did not make such studies on our animals. These are contemplated in future experiments to be made on irradiated animals. The nonprotein nitrogen was not elevated.

In regard to the mast cells and the hyperplasia of them, I have not the slightest idea as to what the hyperplasia of the mast cells means.

In answer to Dr. Brues' question about the breakdown of the roentgen units, no one knows about that. Those recovered on the fourth day received 1300 r., and those on the fifth day, 1500 r. In respect to what Dr. LeRoy said, our confirmation of Allen's work is only partial. We did not directly isolate heparin. We titrated it indirectly with antiheparin agents.

THE PATHOLOGY OF SCHISTOSOMIASIS JAPONICA.* Mark M. Bracken and (by invitation) W. R. Bailey, Jr., Pittsburgh, Pa., and Henry M. Thomas, Jr., Baltimore, Md.

Abstract. During the early days of the occupation of the Philippine Islands by American troops in October and November, 1944, some of the troops were unavoidably exposed to water infested with *Schistosoma japonicum.* Although death rarely occurs in the acute stage of schistosomiasis japonica, 3 of these soldiers died and were examined post-mortem at overseas United States Army hospitals. In addition to the 3 cases studied at autopsy, specimens of acute lesions in the rectum, liver, skin, and brain were secured for biopsy from other patients. The older lesions of the disease, seen in 3 Filipinos who died in an American Army hospital following gunshot wounds, are included for comparison.

In the acute cases in this series ova have been found in the mesenteric lymph nodes, skin, brain, meninges, and adrenal medulla. They have been demonstrated in the late cases in retroperitoneal tissues, kidney, cerebellum, and medulla oblongata. In addition, lesions identical to those in which ova were demonstrated but in which eggs were not found were present in the myocardium.

The early lesions are usually miliary, appearing as yellowish white, caseous nodules or minute abscesses measuring from 0.5 to 10 mm. in diameter. In some of the lesions there is a necrotic zone around viable ova. This necrotic zone is surrounded by eosinophilic leukocytes and fewer neutrophilic leukocytes. As the lesion progresses, it presents central ova, either viable or degenerated with varying degrees of distortion and calcification, epithelioid cells, and fibroblastic proliferation in a richly vascular zone. Frequently the ova are partially or completely surrounded by multinucleated giant cells. The latter are usually of the foreign body type, but they may have the appearance of the Langhans' type. Later the eosinophilic leukocytes decrease in number and lymphocytes predominate.

The earliest lesions may coalesce to form large, irregular areas of necrosis in which are scattered the schistosoma ova. Fibroblastic and capillary proliferation begin early in the peripheral zone of the lesion, and in the older cases fibrosis predominates. The oldest lesions consist of shrunken, calcified ova surrounded by more or less dense fibrous tissue, with a moderate degree of lymphocytic cellular infiltration. In this pathologic picture the early lesions represent an unusual and characteristic reaction to the viable ova with necrosis and eosinophils, and the later lesions represent a foreign body reaction.

CORRELATION OF LABORATORY TESTS WITH THE PATHOLOGY OF SCHISTOSOMIASIS JAPONICA IN AMERICAN SOLDIERS. Stuart W. Lippincott and (by invitation), Lester D. Ellerbrook and Mark Rhees, Seattle, Wash.

Abstract. During the Leyte campaign in the Philippines a number of American soldiers were infected by the cercariae of *Schistosoma japonicum.* A group of 495 of these patients was studied with reference to tests of liver function, various types of stool examination, distribution and fate of antimony during and after treatment, clinical course of the disease, and the results of autopsies on 2 patients dying violent deaths during the period of investigation. A total of 17,295 examinations, using 8 technics, was made on 12,880 stools. The results showed that the direct smear in combination with one or more of the sedimentation methods detected more positive stools than if any one method was used exclusively for examination. In order to detect the maximum number of positive stools, the patient should be followed serially by stool examinations for a period of 10 consecutive days.

The lesions observed in the livers in the 2 autopsies consisted of either minute

* This article will appear in a subsequent issue of *The American Journal of Pathology.*

frank abscesses or fibrotic nodules. There was no indication that at this stage of the disease damage to the liver sufficient to impair its function had occurred. The initial studies of liver function in patients returning to this country from overseas showed the following incidence of abnormal results: globulin, 5 per cent; formol gel, 1 per cent; icterus index, 4 per cent; serum bilirubin, 6 per cent; urobilinogen, 0 per cent; intravenous hippuric acid, 5 per cent; galactose tolerance, 4 per cent; and bromsulfalein retention, 12 per cent. Repeated determinations of the bromsulfalein test and of the serum bilirubin concentration showed a definitely increased incidence of mild abnormalities toward the end of, and following, treatment with trivalent antimony compounds. In a small group of cases followed beyond 90 days, the incidence was markedly decreased.

When approximately 45 mg. of antimony were administered on alternate days, the plasma concentrations increased from about 8 μg. per liter to nearly 85 μg. after 3 weeks of treatment. After discontinuance of treatment the plasma concentrations decreased by 52 per cent in 12 days, and 80 per cent in 28 days. The concentration in blood cells was greater than that of plasma. The daily urinary antimony excretion increased from 2 mg. on the day of the first dose to roughly 10 mg. during the latter part of treatment. Forty days after discontinuance of treatment the daily excretion was still about 1 mg. The average 24-hour excretion in the feces varied from about 0.5 mg. per day during the first week to 2 mg. daily toward the end of treatment. Small amounts were still being excreted 100 days after treatment. The combined excretion in urine and feces toward the end of treatment was roughly 24 mg. in 2 days, or about 55 per cent of the amount administered in the preceding dose.

Tests of liver function performed before, during, and after treatment in these soldiers have shown that successive courses of antimony cannot be given with impunity. Furthermore, gross and histopathologic studies from 2 autopsies showed the hepatic lesions to be circumscribed with no evidence of fibroblastic proliferation, and that, even if antimony treatment failed to kill the worms, it would appear impossible to have enough seeding of the liver with eggs to destroy sufficient parenchyma to produce a true cirrhosis.

Discussion of Papers on Schistosomiasis Japonica

(Dr. H. M. Permar, Pittsburgh, Pa.) When Dr. Bracken sent us his first collection of autopsy sections, there were no data with them. However, we had our new Ash and Spitz, and so, comparing sections and text, we were able to alter our first opinion that we were dealing with tuberculosis in certain sections of the lungs and lymph nodes, and to arrive at the conclusion that we had lesions of schistosomiasis in all of the tissues. The sections are much more like tuberculosis than the areas selected for lantern slides. The important point, it seems to me, in Dr. Bracken's paper is the true nature of the early lesion. It is not easy to decide whether the ovum contains and gives off some toxic substance which causes necrosis, or whether it causes a polymorphonuclear exudate, chiefly eosinophilic, which then undergoes softening and breakdown. I rather incline to the latter view, and I believe Dr. Bracken leans to the former.

(Dr. Michael V. Mackenzie, Boston, Mass.) I should like to ask Dr. Bracken two questions. First, was the lesion which he showed that did not contain an ovum (even after serial sections) the only such lesion encountered in his case? Second, what significance does Dr. Bracken attach to this lesion (or lesions, if similar ones were also found)? Does he believe that it may be entirely a toxic reaction? Dr. Jaffé of Venezuela has reported that in experimental animals which have been infected only by male schistosomes, such lesions can occur. The implication, therefore, is that the presence of an ovum is not necessary for the pro-

duction of such lesions and that the adult schistosomes are truly pathogenic in their own right.

(Dr. Gustave J. Damin, St. Louis, Mo.) Dr. Lippincott has established the need for a large number of stool examinations in ruling out schistosomiasis in patients under treatment. When a concentration test is used, one should be selected which involves the sedimentation of a relatively large amount of feces. The schistosome eggs are classed among the heavier eggs and are not well concentrated by flotation technics.

(Dr. Bracken) In regard to Dr. Permar's question whether necrosis precedes or follows cellular exudate, we have seen in a very early lesion without cellular reaction a radial arrangement of filamentous material extending outward from the wall of a single ovum. This material could possibly be a lytic substance produced by the ovum.

Dr. Mackenzie, did you refer to the extensive lesion in the heart or to the very small one?

(Dr. Mackenzie) The very small lesion, the one which you stated did not reveal an ovum in serial sections.

(Dr. Bracken) It was just by chance that this lesion was present in a block removed from the heart. Perhaps the section not included in the block may have contained an ovum.

HISTOPATHOLOGY OF EXPERIMENTAL LEPTOSPIROSIS. Parker R. Beamer (by invitation), St. Louis, Mo., Hugh G. Grady, Philadelphia, Pa., and (by invitation) H. I. Firminger, Boston, Mass.

Abstract. Leptospirosis was produced in a relatively large series of apparently normal and healthy guinea-pigs weighing between 125 and 250 gm. Rectal temperatures and other clinical data were recorded prior to and during the course of the disease established by intraperitoneal inoculation of widely variable amounts of a culture of *Leptospira icterohaemorrhagiae,* isolated from a wild rat and maintained by animal passage and serial transfers in modified Schüffner's medium. At arbitrarily chosen, successively increasing intervals, animals were sacrificed, some being allowed to recover from the disease in order to study tissue from convalescing animals.

Multiple petechiae and larger foci of hemorrhage, particularly in the lungs and less commonly in the colon and adrenal glands, were observed within 48 hours. On the 4th day of the disease hemorrhagic foci were usually widespread, larger, and more numerous, occurring in the lungs, skin, and subcutaneous adipose tissue, serous membranes, gastrointestinal tract, kidneys, adrenals, epididymis, pancreas, retroperitoneal tissues, myocardium, and skeletal muscles. Cellular infiltration associated with hemorrhagic foci was minimal or absent. On the 5th and 6th days, as temperatures receded, generalized icterus developed. At this time hepatocellular disarray was prominent in the liver; necrosis of hepatic cells, individually or in small groups, and increases in mitotic figures were noted. At about this same period the kidneys were enlarged and pale, and degenerative changes with some necrosis of the proximal tubular epithelium were observed. There were small hemorrhages in a few glomeruli, and the distal and collecting tubules contained albumin and hyaline, cellular, and hemoglobin casts. Tissues from convalescent animals revealed little or no evidence of the disease.

Although hemorrhagic foci occurred in the myocardium, gastrointestinal tract, pancreas, brain, skeletal muscle, ovaries, testes, epididymides, and subcutaneous and retroperitoneal tissues, no leptospirae were demonstrated by silver impregnation, in association with these lesions. Organisms were present in largest numbers in the lungs within 48 hours. By the 4th to 5th days, leptospirae were found in

appreciable but decreasing numbers in the lungs, and the liver contained markedly higher numbers. From the 6th to the 19th days, large numbers were present in the renal tubules, whereas extremely few could be demonstrated in the other tissues.

Discussion

(Dr. Walter H. Sheldon, Atlanta, Ga.) I failed to catch whether you mentioned the animal used for your experiments. I wonder whether the natural course of the disease in your experimental animals showed relapses during the third week of the disease, which so commonly occur in human leptospirosis. Did you have any opportunity to study the changes of leptospirosis in the striated muscle, and were you able to demonstrate the organisms within these lesions?

(Dr. Ralph D. Lillie, Washington, D.C.) I failed to note in Dr. Beamer's presentation mention of the interstitial lymphocytic and other round cell infiltration in the kidney which occurs in human Weil's disease. We have seen it also in the experimental animals which we worked with some years ago.

(Dr. Beamer) In answer to Dr. Sheldon's question, the experimental animal used was the guinea-pig. In the relatively short observation period, no relapses were seen. We failed to find the lesion which you reported in striated muscle in human beings. I think, however, the course of the disease in our experimental animals was too acute to find degeneration and the subsequent changes which have been noted in human beings. We did find foci of hemorrhage in the muscles, and in the animals which recovered from the disease we found no additional changes during the relatively short period of observation. In a few instances in which the attempt was made, leptospirae were not found in the muscular lesions.

In answer to Dr. Lillie's question concerning the infiltration of cells in the kidney, that was noted in many instances, but usually not to a significant degree. We found, in general, that interstitial infiltration in the kidney was only of slight degree in these animals, and infiltration of similar extent was found in an appreciable number of control animals of comparable ages.

THE RECIPROCAL RELATIONSHIP OF SURFACE TEMPERATURE AND TIME IN THE PRODUCTION OF HYPERTHERMIC CUTANEOUS INJURY. A. R. Moritz and (by invitation) F. C. Henriques, Jr., Boston, Mass.

Abstract. The threshold for the occurrence of irreversible epidermal injury at surface temperatures varying between 44 and 100° C. was observed in porcine and human skin. Surface temperature was maintained at a constant predetermined level by contact with a rapidly flowing stream of hot liquid. The time required to produce irreversible injury bore an inverse relationship to temperature. The effect of circulation of blood through the dermal capillaries on the susceptibility of the skin to thermal injury was investigated. The causation of rapidly fatal circulatory failure incident to generalized cutaneous exposure to excessive heat was determined.

Discussion

(Dr. Raymond H. Rigdon, Little Rock, Ark.) What was the age of the animals you used, and was the hair removed before you made these observations?

(Dr. Herbert Lund, Cleveland, Ohio) Was there a difference in the injury of the deeper tissues at different temperatures? Cooking a roast beef at low temperature for a long time produces changes throughout most of the meat, and we know that the same effect is not produced by a flash of heat of high intensity. In these injuries of the skin I would be interested to know if a long duration of low temperature heat produces a deeper effect.

(Dr. Moritz) The pigs we used weighed between 10 and 15 kg. and it was found that the lateral thoracic and lateral abdominal areas were uniformly responsive.

There are other sites that do not respond uniformly, and which should be avoided. The pigs were clipped, although this is not necessary.

Dr. Lund's question has to do with the establishment of gradients through the skin, and time did not permit me to discuss the measurement of skin gradients. The higher the surface temperature, the steeper the trans-cutaneous gradient becomes and the shorter the time required to destroy the epidermis. As surface temperature is increased, the damage to the deeper tissues becomes relatively less severe than that to the superficial cells. You can char the surface of the skin with no change in the dermis, if you are using a high enough temperature. The long exposure time required to kill the epidermal cells at the lower temperatures predisposed to a greater depth of injury.

QUANTITATIVE HYPOTHERMAL PRODUCTION OF CLOSED CEREBRAL INJURY. G. M. Hass and (by invitation) C. B. Taylor and J. E. Maloney, Chicago, Ill.

Abstract. A method for producing quantitative controlled destruction of local areas of the cerebral cortex of rabbits by the use of a freezing device applied to the external surface of the intact skull is described. Cylindrical lesions of the cortex varying from 2 to 25 mm. in diameter and 1 to 7 mm. in depth, even extending through the white matter into the lateral ventricles, may be produced. The breadth and depth of the lesions depend upon the dimensions of the tip of the freezing device and the duration of contact of the tip of the device with the skull. The lesions resemble strictly localized infarcts or contusions of the brain. In single acute experiments, survival of the animal depends upon the volume and depth of the lesions. By production of successive sublethal lesions, over 30 per cent of the brain can be progressively destroyed without opening the skull, or introducing any factor such as widespread hemorrhage or concussion. An experimental approach to therapy of closed intracerebral hemorrhage with necrosis of the brain by a quantitative method becomes possible.

Discussion

(Dr. A. R. Moritz, Boston, Mass.) I would like to inquire as to the mechanism of the injury. Is this tissue frozen? Does it die because of intracellular congelation, because of interference with circulation, or is there some other injurious mechanism?

(Dr. Jacob Werne, Jamaica, N.Y.) I wonder whether the exact location of the traumatic lesion was considered in evaluating these experiments. In medicolegal practice it is well known that the site, no less than the extent of the lesion, frequently has a bearing on the outcome of a given case.

(Dr. Alfred Angrist, Jamaica, N.Y.) Is there any evidence, in the clinical sense, of increased intracranial pressure in these animals? In other words, is the degree of edema sufficient to alter the dynamics of the cerebrospinal fluid, and were any changes found in the pons in these animals? With human head injuries it is well known that about one-third of the cases will show pontine hemorrhages.

(Dr. Taylor) The tissue has been destroyed primarily by the low temperature. The cooling plate has a temperature of $-50°C$.

In reply to Dr. Werne, there is very little hemorrhage in the damaged tissue, and very little reaction around the lesions. All damage was confined to the cerebrum in this group and there was no relation between the site of injury and death. There was no difference whether the lesion was on the left or the right side. If lesions extended into the midbrain, animals usually did not survive. We confined our studies to the cerebral cortex for that reason.

Dr. Angrist asked about increased intracranial pressure. We have not been able to measure the cerebrospinal fluid pressure in rabbits. Animals did exhibit symptoms of increased intracranial pressure. We have found no changes in the pons.

We have had a few rabbits with a very small amount of subarachnoid hemorrhage, just over the fourth ventricle.

THE DISTRIBUTION OF BRAIN STEM LESIONS IN POLIOMYELITIS. John C. McCarter and (by invitation) M. Barnhart, R. Rhines, and H. W. Magoun, Evanston, Ill.

Abstract. This report is concerned with the detailed anatomic distribution of the lesions of acute poliomyelitis in human brain stems, and the correlation of clinical symptoms. The material of the inquiry was 7 brains and cords obtained at autopsy from patients dying of acute poliomyelitis at Evanston Hospital in the epidemics of 1943–44–45. Sections from several areas of cerebral cortex, cerebellum, and spinal cord, together with serial sections of the entire brain stem, have been examined, and the distribution of lesions plotted in each case. All 7 cases (6 of bulbar type and 1 of spinal type, clinically) had severe damage to the reticular formation of the brain stem. This constant pattern of involvement is regarded as of prime importance in the causation of symptoms of respiratory impairment, vasomotor collapse, and muscle spasticity exhibited by patients dying of acute poliomyelitis.

THE CHANGES IN THE MOTOR CORTEX IN ACUTE POLIOMYELITIS. Kornel L. Terplan, Buffalo, N.Y.

Abstract. In 49 of 56 cases of acute poliomyelitis, distinct recent changes were found in the motor area, particularly in the third to sixth layer, occasionally extending into the adjacent subcortical white matter. These changes were readily seen with the naked eye in preparations stained with thionin or toluidin blue, and, in particular, small nodular infiltrations and micro-abscess-like lesions about dead or necrobiotic nerve cells. Histologic analysis revealed the same type and frequently the same intensity of cellular damage and inflammatory reaction as in the anterior horns of the spinal cord and in the brain stem. The degenerative changes involved the pyramidal cells in the cortex, including the Betz cells, leading occasionally to complete disappearance of giant pyramidal cells and their replacement by small triangular glial nodules. There was an excessive activation of microglial cells which, together with disintegrating leukocytes, formed the usually ill defined abscess-like lesions. In some cases there was band-like vertical infiltration extending upward into the second or, rarely, into the first layer. In other cases there was more diffuse proliferation of microglial cells and extensive neuronophagia. Perivascular cuffing in the motor cortex and the adjacent subcortical white matter was a prominent feature. In a few instances there were small areas of recent softening with masses of fat granular cells about veins in the fifth and sixth layer of the cortex, accompanied by segmental disappearance of all nerve cells in this area. All changes were most pronounced in the posterior portion of the precentral gyrus. The various reactive inflammatory changes in the supporting mesodermal and ectodermal tissue were similar to the acute inflammatory response in the anterior horn of the spinal cord, medulla, dentate nucleus of the cerebellum, tegmental portion of the pons, and in the subthalamic and thalamic area. Various representative areas from other parts of the pallium were examined, including the frontal, occipital and parietal lobes, Ammon's horn and hippocampal gyrus, and the island of Reil, and no similar cortical changes were seen. In comparison to the distinct damage revealed in the motor cortex, the remainder of the pallium could be considered as negative.

This selectivity of the poliomyelitic virus for the neurons of the motor cortex appears just as characteristic in our experience as the marked damage of the nerve cells in the anterior motor horns. As far as the pallium is concerned, it is diagnostic of acute poliomyelitis. Selectivity for the motor cortex has been observed following

intracerebral inoculation of the monkey with the human virus by Hurst, Luhan, and Peers. Findings apparently identical with ours had been observed in human poliomyelitis by Andre-Thomas and Lhermitte, by Spielmeyer, and especially by Koernyey.

In the 7 remaining cases there were only minimal findings in the motor cortex, including neuronophagia about a few small pyramidal cells and very slight pericapillary round cell infiltration. In all 56 cases acute poliomyelitic damage was present at various levels of the spinal cord, in the medulla and pons, and in those areas of the peduncles, hypothalamus, globus pallidus, and thalamus which usually are found involved in acute poliomyelitis.

The selective involvement of the motor cortex was observed not only in the majority of the cases from the epidemic in Buffalo in 1944, but also in isolated cases occurring in the years 1937, 1939, 1941, 1943, 1945, and 1946.

Discussion of Papers on Poliomyelitis

(Dr. Russell L. Holman, New Orleans, La.) I would like to ask Dr. McCarter the age of the patients and the duration of the disease; also whether there was any clinical correlation with the distribution of the lesions.

(Dr. McCarter) First, as to the age of the patients: the youngest in this series was 2½ years, the oldest was 46, with a large proportion of the entire 18 cases ranging below 20 years. As to the time interval from the onset of the disease to the time of death, that varied between approximately 36 hours in the shortest case, to 23 days in the longest one. I did not quite understand your second question.

(Dr. Holman) Was there any correlation between the duration of illness, clinical symptoms, and the type of death and the location and extent of the lesions?

(Dr. McCarter) I think there was, very definitely. We were impressed with the large proportion of these cases which showed circulatory collapse, which showed respiratory collapse, pulmonary edema, and lack of tonus of the gastrointestinal tract. In these fatal cases these symptoms were much more prominent than in the ones who survived, my clinical colleagues tell me, but many of those who survived showed varying degrees of these symptoms.

POST-MEASLES ENCEPHALOPATHY. Karl T. Neubuerger, Denver, Colo.

Abstract. The pathologic picture of acute post-measles encephalitis has been established, but lesions of the brain in more chronic cases have been reported in only a few instances. The findings in 2 patients who survived the acute stage are presented. The first case was that of a 60-year-old man who had measles and, 10 days later, lapsed into a semistuporous state with generalized spasticity and progressive mental deterioration. He died 10 weeks after onset of the cerebral symptoms. The second case was that of a 7-year-old boy who became semicomatose, with fever, rigidity, and shaking, 10 days after having been taken ill with measles. He died 6 weeks later. The immediate cause of death in both patients was bronchopneumonia. The nervous lesions were similar. The essential site was the cerebral white matter where patchy, sometimes coalescing foci of perivenous demyelination were found. The axis cylinders were damaged to a less extent than the myelinated fibers. The foci were not well defined; some were coarsely vacuolated, particularly in the older patient. Proliferated microglial cells were laden with lipoid disintegration products of the destroyed nerve fibers. In some fields, areas of softening with densely packed gitter cells were observed. Large astrocytes were present within the foci and in their immediate vicinity. In what appeared to be older lesions they had superseded the microglial elements. Glial fibrils forming a coarse network were numerous in the older case, but sparse in the younger one. Some of the intrafocal veins exhibited stasis or recent thrombosis. In addition to the changes described, the brain of the boy showed a few scattered foci

with plasmatic gliosis in the extracortical gray matter, with very little damage to the nerve cells. There was also some marginal gliosis in pons, medulla, and upper cord.

As to the pathogenesis of this condition, the following statement can be made. In a limited number of patients with measles the causative agent, possibly a virus, reaches the brain probably by way of the blood stream, and displays its activity mainly in the white matter which is less well vascularized than the gray. It causes disturbances in the terminal circulation, particularly on the venous side. Apparently it diffuses through limited perivenous zones only. It is possible that allergy plays some part since experimentally produced allergic lesions have similar histologic features.

The relation of post-measles encephalitis to multiple sclerosis is as yet an unsolved problem. The findings in the cases of encephalopathy under discussion do not favor the assumption of the identity of both diseases, despite many similarities. Some of the features present in post-measles encephalopathy but not in multiple sclerosis are lack of predilection for the periventricular region; scarcity of cortical foci; strictly perivenous location; lack of sharp demarcation of the foci; more damage to the axis cylinders; occurrence of fibrillary gliosis only in the later stages; lack of "advancing cell wall," inflammatory features, and mesenchymal proliferation as seen in recent foci of multiple sclerosis; absence of appreciable variation in the age of the foci; and definite difference in the clinical picture.

Discussion

(Dr. John C. McCarter, Evanston, Ill.) What symptoms in the clinical course of these patients were ascribed to post-measles encephalitis? They died, of course, of bronchopneumonia, which I assume was not related to the disease.

(Dr. Neubuerger) The bronchopneumonia was not related to the disease. The main symptom was the very marked spasticity in both cases.

HISTOLOGY OF CORONARY ARTERIES IN NEWBORN INFANTS. R. J. Fangman (by invitation) and C. A. Hellwig, Wichita, Kans.

Abstract. In a recent paper, William Dock suggested that the preponderance of males dying of coronary disease might be explained by inherited variations in the thickness of the coronaries. He found the intima of coronary arteries to be much thicker in newborn males than in females. Dock concluded that because of this anatomic peculiarity it has already been determined at birth whether coronary occlusion may occur.

We examined during the past year the coronary arteries of 30 newborn infants. Sections were made from the three main vessels of 15 males and 15 females. The frozen and paraffin sections were stained by different methods in order to study not only the cellular and fibrous elements, but also accumulations of lipoid and hyaline material. In 12 cases, 8 males and 4 females, we found thickening of the intimal layer most often in the anterior descending coronary artery, but never involving the total circumference of the vessel. The cushion-like elevations varied from small buds to more than one-half of the circumference of the vessel. Verhoeff's stain for elastic fibers revealed small breaks in the internal elastic lamella, or it was composed of frayed elastic fibers. In some cases it was split into two layers and under several elevations the elastic lamella was absent. No less revealing were the sections stained with sudan. We found fine deposits of lipoid along the elastic fibers and in the stroma of the cushions and sometimes also in large histiocytes.

While our findings are identical with those of Dock, concerning the presence of cushion-like thickening of the coronary arteries in newborn infants, our interpretation differs from his view that these are inherited anatomic peculiarities. The

presence of lipoid deposits and the destruction of elastic tissue throw serious doubts on the explanation offered by Dock. We believe that these are pathologic processes, *i.e.*, the earliest stages of intimal arteriosclerosis. Our standpoint is supported by the facts that the thickenings are restricted to certain areas of the intimal circumference, that they are most common in the anterior descending branch, and that they are more often found in male infants, characteristics which apply to coronary sclerosis of the adult as well.

The degeneration of the elastic fibers under the cushions is caused most likely by the deposits of lipoid. According to Hueper, deposits of macromolecular colloids in tissue interfere with the normal exchange of nutritive substances between tissue and plasma. The proliferation of fibroblasts is then a reparative process following injury of the tissue by accumulation of lipoid. We regard the precipitation of lipoid as the primary event in the formation of these cushions.

Discussion

(Dr. *Theodore J. Curphey, Hempstead, N.Y.*) I should like to ask whether these changes were more noticeable in the proximal third of the anterior descending branch than in the rest of its course. In many cases of sudden death secondary to coronary occlusion, we have seen the occluding arteriosclerotic process confined to the first 2 or 3 cm. of the anterior descending branch.

(Dr. Hellwig) We found most of the cushion-like elevations in exactly those locations which Dr. Curphey pointed out. Only in the epicardial portions of the coronaries, never in the muscular branches, do these lesions occur.

(Dr. *Russell L. Holman, New Orleans, La.*) I would like to ask if you found these changes in other vessels, particularly around the aortic valves and around the mouths of the coronary vessels; also whether there was any correlation between the deposits in the coronary vessels and elsewhere in the large vessels.

(Dr. Hellwig) We did not search systematically for lipoid deposits in other blood vessels, and I am unable to answer Dr. Holman's questions. However, in former studies on the atheromatosis of the mitral valve, I noticed distinct lipoid spots in the mitral valve already in the second month of life.

(Dr. *S. Milton Rabson, Fort Wayne, Ind.*) What was the relationship between the disease from which the newborn infants died and the degree of severity of the process described?

(Dr. Hellwig) In our positive cases the cause of death varied. We were not able to establish any relationship between cause of death and presence or degree of lipoid deposits in the coronaries.

(Dr. *Howard T. Karsner, Cleveland, Ohio*) Do you attach any significance to the difference in the incidence in the two sexes which you have observed?

(Dr. Hellwig) I believe that the sex difference is significant. The view of Dock that males have a slower pulse and higher stroke volume seems supported by the fact that in male newborns the weight of the heart is slightly higher than in females. The predilection of these lipoid deposits for the anterior descending coronary of males could well be explained by a more vigorous vibration of the vessel wall during systole disturbing the colloidal stability of the lipoid dispersion in the intima.

THE EFFECT OF PATENT DUCTUS ARTERIOSUS ON THE DEVELOPMENT OF PULMONARY VASCULAR LESIONS.* Thomas D. Kinney and (by invitation) Kenneth J. Welch, Boston, Mass.

Abstract. The effect on the pulmonary vascular bed of an abnormal communication between the systemic and pulmonic circulation was studied in a group of patients having uncomplicated patent ductus arteriosus. No pathologic lesions in the small branches of the pulmonary artery were observed in 27 patients with

* This article will appear in a subsequent issue of *The American Journal of Pathology.*

uncomplicated patent ductus arteriosus. This evidence suggests that the belief in the common occurrence of pulmonary vascular disease resulting from a patent ductus arteriosus is erroneous.

MYOCARDITIS IN EARLY LIFE. Reginald K. House (by invitation), Hanover, N.H.

Abstract. The gross and microscopic findings of 5 cases of myocarditis of obscure origin in infants are described. The various etiologic factors are mentioned, with emphasis on possible hypersensitivity or virus infection.

Discussion

(Dr. I. Davidsohn, Chicago, Ill.) I did an autopsy just a few weeks ago on a baby, age 26 hours, in whom I found an acute diffuse myocarditis. The baby was born after an uneventful pregnancy, and showed, since birth, clinical manifestations that were interpreted as meningeal hemorrhage. No other lesions were found at autopsy. The endocardium was normal; the myocardial lesions were visible to the naked eye through the epicardium as distinct yellowish gray areas, mainly on the anterior surface of the left ventricle.

CONGENITAL HEART DISEASE WITH NECROTIZING ARTERITIS (PERIARTERITIS NODOSA) LIMITED TO THE PULMONARY ARTERIES: REPORT OF CASE WITH NECROPSY. Jacob W. Old (by invitation) and William O. Russell, Santa Barbara, Calif.

Abstract. An 11-year-old California boy of Mexican parentage, known to have congenital heart disease from birth, died after an illness of 30 days. He had enjoyed good health until his terminal illness, except for moderate limitation of physical activity imposed by his disease. His principal symptoms were attacks of headache, malaise, and on two occasions a slight elevation of temperature. Terminally, there was excessive perspiration and a dry cough, and a tentative diagnosis of pneumonia was made. Treatment with oral penicillin was unsuccessful because of vomiting. Bright red blood, presumed to be of pulmonary origin, found on his clothing led to hospitalization in a tuberculosis sanatorium. Physical examination revealed a lethargic, cyanotic boy with rapid pulse and respirations and a diastolic apical heart murmur. A roentgenogram of the chest showed soft infiltrations, varying from 1 to 5 mm. in diameter, in all pulmonary lobes. The family and personal histories disclosed no known sensitizations or symptoms of allergic states, and there was no known therapy with sulfonamide drugs. A polymorphonuclear leukocytosis of 20,000 was present before death.

Necropsy revealed a large defect in the membranous part of the interventricular septum with marked hypertrophy and dilatation of the right ventricle, and moderate chronic mitral endocarditis. In the lungs the medium-sized arteries contained recent and partially organized thrombi with a moderately broad zone of induration surrounding the involved vessels. Microscopic studies of the lungs showed acute necrotizing arteritis of the arterioles and of the medium and small arteries with hyalinization and fibrinoid change in the wall accompanied by infiltrations of polymorphonuclear leukocytes. The most acute lesions were frequently limited to focal necrotic changes in the arterial wall. Generally, however, there was complete necrosis of the arterial walls in which the lumina were filled with thrombi, occasionally showing organization. The arterial walls and adventitial tissues were infiltrated with polymorphonuclear leukocytes and macrophages but no eosinophils. Some of the arteries showed organized thrombi with recanalization. Arteries and arterioles in sections taken from all other viscera except the brain, which was not examined, were normal. The necrotizing arteritis was regarded as characteristic of the lesions observed in periarteritis nodosa occurring in man and experimentally produced in sensitized animals.

The localization of the arteritis to the pulmonary arteries was explained on the basis of a greater exposure of these arteries to an unidentified agent causing the

arterial disease of periarteritis nodosa. The defect in the interventricular septum caused a proportionately greater circulation through the right side of the heart and pulmonary arteries than through the systemic arterial system. This case suggests that the blood concentration of the unidentified agent producing necrotizing arterial disease is an important factor in the genesis of the lesion and that a critical concentration must be reached to produce the disease.

Discussion

(Dr. Howard T. Karsner, Cleveland, Ohio) I would like to put this in the form of a question, although it might seem that I am delivering a lecture on the subject of arteritis. It seems to me that if we call all forms of acute arteritis "periarteritis nodosa," we are probably confusing ourselves, or at least not permitting ourselves to be specific in the identification of arterial lesions. In my opinion this case does not fulfill the usual criteria that we apply to periarteritis nodosa, which is a systemic disease. To be sure, its manifestations may be more pronounced in one situation than in another, but even temporal arteritis is now known to be something more than a local disease. I think we should pay some attention, although this is purely a personal opinion, to the infiltration of eosinophils in the exudate. In this particular instance there was no eosinophilia of the circulating blood and, as far as I can learn, there was no eosinophilia in the exudate. Whether we argue for or against hypersensitivity or allergy in connection with periarteritis nodosa, nevertheless it is a widespread disease. We realize, however, that in rheumatic vascular disease there may be a much greater degree of localization. We see it in the myocardium in outspoken, florid rheumatic fever, and in the myocardium lesions are found which are microscopically identical with those exhibited in this case. They occasionally occur in the brain, and of course we all have in mind the discussion concerning the manifestations of rheumatic fever in the lung. With this preliminary lecture, I ask why this should not be classified simply as rheumatic vascular disease in the lung.

(Dr. Old) In our study of the literature of this subject we were first impressed with the fact that there is great overlapping of the lesions of rheumatic disease, periarteritis nodosa, and other forms of arteritis. It was for this reason that the term necrotizing arteritis was used and periarteritis placed in parentheses in the title. This seemed the most logical way to regard these lesions until more definite and specific diagnostic criteria are available for the anatomic diagnosis of rheumatic arteritis. We do not feel that the question can be answered categorically. Against a rheumatic type of arteritis is the fact that no evidence of active rheumatic disease was found in the heart or other organs. We should like to emphasize that this case was presented merely as one in which there was evidence of previous rheumatic infection and in which necrotizing arteritis occurred in only one organ. The localization of the lesions in arteries previously altered as a result of the patent interventricular septum, changes that Dr. Kinney has so well described this afternoon, is an interesting observation that we hoped would stimulate further comment for our better understanding of the unknown factors·giving rise to them.

CLINICOPATHOLOGIC AND EXPERIMENTAL OBSERVATIONS ON THE PATHOGENESIS OF RUPTURE OF THE HEART DUE TO MYOCARDIAL DAMAGE. W. C. Thomas, Winston-Salem, N.C.

Abstract. In addition to the anatomic considerations of size, site, age, and histologic type of infarction, there are numerous physiologic factors concerned in rupture of the heart. Some of these are intracardiac pressure, tensile strength of the wound produced by the injured muscle, and the contractility of the adjacent undamaged musculature. An analysis of the relationship of these various factors and the clinicopathologic findings was made.

Attempts were made to determine the influence of various forms of exertion in

the production of heart rupture. Myocardial damage was produced in rats by searing the anterior surface of the left ventricle. An experiment was carried out in which one group of animals was exercised forcibly by swimming, while another group was allowed to exercise voluntarily. No difference was noted in the incidence of heart rupture in the two groups. Another series of animals was studied to determine the influence of varying obstruction to the inspiratory and expiratory phases of respiration. No ruptures of the hearts occurred even though the period of maximum occurrence served as the time of testing. Finally, the amounts of intraventricular pressures necessary to cause ruptures of the hearts were measured on the tenth day following muscle damage. The pressures varied between 150 mm. and over 400 mm. of Hg. The average pressure was found to be 225 mm. of Hg.

Discussion

(Dr. Howard C. Hopps, Oklahoma City, Okla.) No mention was made of the extent or the type of mural thrombus that might follow infarction and thus reinforce the weakened region. I wonder if that might not be an anatomic factor in protecting the heart from rupture.

(Dr. Jesse E. Edwards, Rochester, Minn.) I would like to ask Dr. Thomas whether he made any correlation between the amount of necrosis and the incidence of rupture. The point I have in mind is whether the necrosis extended to the endocardium. I think every one of us here has seen that in most instances of rupture of the heart of human beings the myocardial necrosis is extensive and frequently involves the muscle down to the endocardium, in contrast to the usual myocardial infarction in which there is no infarct in the subendocardial muscle.

(Dr. Thomas) In reply to Dr. Hopps' remarks, I wish to say that I encountered no mural thrombi in the hearts of these animals.

In connection with Dr. Edwards' question I found that there was generally more extensive necrosis and less connective tissue response in the hearts which ruptured. In these cases the necrosis extended more deeply into the subendocardial tissues than in the hearts which did not rupture.

OBSERVATIONS ON THE LUNGS OF THE NEWBORN. J. Edgar Morison (by invitation), Belfast, Ireland.

Abstract. The morphologic state of the lungs at birth may influence the postnatal picture, but, while considerable variation in the degree of expansion of the air spaces *in utero* has been found, the normal range of variation is not known. In the production after birth of the irregular pattern of initial atelectasis, the importance of variations in fluid absorption from the peripheral parts of the respiratory system and of irregular air entry is emphasized. Secondary edema may further modify the picture. The distribution of edema fluid in the interstitial tissue of the partitions between the air spaces, as studied in the lungs of premature infants, suggests that normally the air spaces are separated from the mesenchyme by a continuous membrane. Cellular reactions in the lung in autopsies from before and after the era of specific chemotherapy have been studied with special reference to mononuclear reactions. Reactions actually in the interstitial tissue are slightly more marked in some of the recent cases, but the significance of this is still uncertain.

THE PATHOGENESIS OF CONGENITAL POLYCYSTIC LUNG AND ITS CORRELATION WITH POLYCYSTIC DISEASE OF OTHER EPITHELIAL ORGANS. RECONSTRUCTION OF CYSTIC ELEMENTS IN TWO CASES.* Robert F. Norris and (by invitation) Ralph M. Tyson, Philadelphia, Pa.

Abstract. The polycystic lesions in the lungs of 2 infants are described and reconstructions of the cystic elements are illustrated. The fundamental lesion ap-

* This article will appear in a subsequent issue of *The American Journal of Pathology.*

pears to be focal segmentation preceded or followed by focal dilatation of the small bronchi and bronchioles. If the disease becomes arrested in the stage of focal dilatation without segmentation, bronchiectasis results which may persist for the duration of life. When the bronchi are broken up into isolated segments, some of these segments persist as gradually enlarging cysts. The well known lesions of polycystic disease are thus established. The lesions and sequence of anatomic changes in the polycystic lung are similar to those previously described in the polycystic kidney, liver, and pancreas. In general the lesions of polycystic epithelial organs primarily affect the nonfunctioning system of ducts. By analogy with the process of physiologic resorption in normally provisional organs or parts of organs, it is concluded that polycystic disease is a pathologic manifestation of normal fetal resorption and degeneration. The polycystic organ is therefore partially provisional. The fundamental defect which initiates the developmental anomalies is unknown and the etiologic importance of anomalies of circulation in polycystic organs has not been determined.

SPONTANEOUS AND INDUCED GLOMERULONEPHRITIS IN AN INBRED STOCK OF MICE. Arthur Kirschbaum (by invitation) and E. T. Bell, Minneapolis, Minn.

Abstract. Mice of the NH strain develop glomerulonephritis spontaneously. The structural changes in the glomeruli closely resemble those found in human glomerulonephritis. In the acute stage, which may be seen in some kidneys, the capillaries are filled with endothelial cells. The majority of the affected glomeruli are in the chronic stage and show endothelial proliferation with thickening of the intercapillary septa. Associated with the renal lesions are albuminuria, edema, low plasma protein levels, nitrogen retention, anemia, and occasionally hypercholesterolemia.

This stock of mice developed glomerular lesions following the administration of urethane (1 mg. per gm. of body weight in 10 per cent aqueous solution administered intraperitoneally once a week for 4 to 6 months). The glomerular lesions were not identical with those of human glomerulonephritis, but resembled those of lipoid nephrosis in that there was thickening of the capillary basement membrane and only a moderate endothelial proliferation. There were numerous thromboses in the glomerular capillaries. The urethane-injected animals with advanced glomerular lesions were edematous, exhibited albuminuria, low levels of plasma proteins, and renal insufficiency (elevation in blood urea nitrogen).

THE JUXTAGLOMERULAR APPARATUS IN EXPERIMENTAL HYPOTENSION. F. W. Dunihue (by invitation), Burlington, Vt.

Abstract. The function of the juxtaglomerular apparatus (JGA) is an open question; yet there is considerable indirect evidence in support of Goormaghtigh's hypothesis that the cells of the JGA secrete renin. The principal basis of this hypothesis is that the increased renin activity found in the early stages of experimental renal hypertension is accompanied by hypertrophy of the JGA, and, in most animals, by an increase in fuchsinophilic granular cells. Since an increased renin activity has been reported in a variety of hypotensive states, such as hemorrhagic shock and adrenal insufficiency, it is to be expected on the basis of the above hypothesis that the JGA would be hypertrophied and contain increased granular cells in these conditions. The experiments here reported are in general agreement with this expectation.

Hemorrhagic shock was induced in 20 rabbits using Wolcott's method. Fatal shock under these conditions produced changes in the JGA which varied with the duration of the shock. In rabbits dying within 2 to 5 hours, there was an increase in the granular cells of the apparatus; the granules were characteristically finer, less numerous, and stained differently than those seen subsequent to procedures which produce experimental renal hypertension. In fatal shock of shorter duration

the increase in granular cells was not so clear, but the number of such cells in all animals was slightly above or at the upper normal limits.

Bilateral adrenalectomy was performed in two stages in rabbits, cats, dogs, and monkeys. The JGA in all these animals was hypertrophied, hyperplastic, and contained increased fuchsinophilic granular cells, which morphologically and tinctorially were similar to those seen in experimental renal hypertension.

The stimulus provoking these reactions in the JGA cannot be solely hypotension, since hypoxia also was present. However, the fact that these changes were present in adrenalectomized monkeys prior to an actual drop in the blood pressure and in rabbits which show no signs of deficiency suggests hypoxia or some other factor as the effective stimulus.

It is not clear at the present time whether these juxtaglomerular changes can be related to VEM which is reported to be of renal cortical origin by Shorr and his colleagues.

Discussion

(Dr. N. Goormaghtigh, Ghent, Belgium) I think it is only natural that I should comment on the work of Dr. Dunihue, and that I should congratulate him for the work he has done. In research there is always a human side, and it is very gratifying for a research worker to find that somebody has confirmed and extended the observations which he has made originally. I should like to say that those who are interested in the problem can very well follow the technic used by Dr. Dunihue. I think his technic is better than mine, and it is advisable that his directions should be followed. I should like also to emphasize the importance of the factor which he has underlined, namely, renal anoxia as a cause of the increase of the arteriolar secretory cells.

THE PERIODIC ACID ROUTINE APPLIED TO THE KIDNEY.* J. F. A. McManus (by invitation), Birmingham, Ala.

Abstract. Selective staining of the basement membrane of the renal tubules and glomerulus of the normal kidney is produced by treating sections with periodic acid and then with Schiff's reagent for aldehydes. In abnormal kidneys there is coloring not only of the basement membrane, but also of the hyaline droplets in the tubular epithelium, the hyalin of arteriolosclerosis, and the granular cells of the renal arterioles. This coloring of basement membrane and other structures in the kidneys may depend upon the production of aldehyde by periodic acid from the carbohydrate moiety of mucoprotein, since periodic acid has the ability to produce aldehyde from carbohydrate. However, histochemical interpretations should be deferred, because serine, threonine, and hydroxylysine also form aldehyde when acted upon by periodic acid and because many compounds of carbohydrate joined to proteins or to lipids are present in tissues.

Discussion

(Dr. N. Goormaghtigh, Ghent, Belgium) I should like to ask if the use of periodic acid gives any indication of the chemical structure of the granules found in the cells of the juxtaglomerular apparatus.

(Dr. McManus) Two groups of materials are known to color with Schiff's reagent after periodic acid: (1) Carbohydrates, either in combination with protein or polymerized, and (2) three amino acids that Nicolet and Shinn have found. However, the chemists think that these three amino acids will have to be in a terminal position in order to be attacked by periodic acid, so it is quite within the realm of probability that a carbohydrate or a carbohydrate compound is being demonstrated in the granular cells of the renal arterioles.

Since preparing this paper we have been able to find the renal arteriolar granular

* This article will appear in a subsequent issue of *The American Journal of Pathology.*

cells in a wide variety of conditions which we are not yet able to explain fully. Apparently in certain conditions autolysis is not so rapid as it is in others, as regards the granular cells, so with this method the survey of many kidneys may be worth while.

THE PANCREAS IN UREMIA. Archie H. Baggenstoss, Rochester, Minn.

Abstract. Histologic examination of the pancreas at necropsy in cases of uremia frequently revealed varying degrees of dilatation of the acini, flattening of the lining epithelial cells, and inspissation of intra-acinar secretion. Investigation showed that the lesion was present throughout the pancreas and was not associated with obstruction of the ducts. Cells of the involved acini had lost their zymogen granules and stained uniformly pink with hematoxylin and eosin. The material in the acini appeared amorphous, stringy, or laminated and also stained pink with this stain. It reacted like protein to a variety of stains. It occasionally contained cellular débris. When a half or more of the acini were involved, the lesion was classified as severe; when many, but less than half, of the acini were involved, the lesion was considered as moderately severe; and when only a few acini in each lobule were involved, the lesion was classified as mild.

The lesion was found in 33 (39 per cent) of 85 cases in which chronic glomerulonephritis terminated in uremia. The lesion was classified as mild in 19 (58 per cent) of the cases, as moderately severe in 9 (27 per cent) of the cases, and as severe in 5 (15 per cent) of the cases. The lesion was found in 36 (42 per cent) of 85 cases in which hypertension terminated in uremia. The lesion was classified as mild in 22 (61 per cent) of the cases, as moderately severe in 12 (33 per cent) of the cases, and as severe in 2 (6 per cent) of the cases. The lesion was found in 52 of 100 cases in which uremia resulted from miscellaneous causes, such as urinary obstruction, pyelonephritis, and extrarenal factors. The lesion was classified as mild in 28 (54 per cent) of the cases, as moderately severe in 16 (31 per cent) of the cases, and as severe in 8 (15 per cent) of the cases.

Neither age, sex, duration of the uremia, nor the degree of azotemia appeared to play a significant rôle in the production of the lesion. The incidence of uremic pericarditis was significantly higher in the group of cases in which the lesion was present. A mild or moderate form of the lesion was present in 40 (20 per cent) of a control series of 200 cases. Although uremia was not listed as a contributory cause of death in any of these cases, azotemia had been present in 10 of the 40 cases. The most common cause of death was intestinal obstruction. Although the cause of the lesion is not clear from this study, it is suggested that dehydration and some as yet unknown metabolic disturbance result in inspissation of secretion and intrinsic obstruction of the pancreatic ductules and acini.

Discussion

(Dr. Tracy B. Mallory, Boston, Mass.) I became very much interested in this lesion which Dr. Baggenstoss has just described and illustrated when I was with the Army in Italy. I find it comparatively uncommon in the material from civilian hospitals whereas I was quite startled by its frequency, which I would estimate at 10 per cent, in the material passing through my laboratory in Italy. Like Dr. Baggenstoss, I observed it in patients dying from renal insufficiency. I noted it, however, with equal frequency and severity in patients with severe infections, particularly peritonitis. The most marked examples which I saw occurred in typhus fever, and all of 24 typhus cases showed the lesion in some degree. We were unable to discover any promising clues pointing toward its etiology, but strongly suspected dehydration. I never found the lesion in any case of sudden death or, in fact, in any individual who had not been severely ill for at least 4 days, usually 6 or more, before death.

(Dr. Theodore Gillman, Chicago, Ill.) The lesion which has just been described

is of great interest to us, because my brother, Dr. Joseph Gillman, and I have been able to produce a very similar reaction in rats by nutritional means. We have also encountered a similar lesion with fibrosis in the pancreas of human subjects who have died with severe malnutrition. In rats the lesion is preceded by, and associated with, severe liver disease, and whether this liver disease plays a direct part remains to be determined. Dorothy Andersen, in her classic work on cystic fibrosis of the pancreas, referred to liver disease being present. Moreover, an examination of her protocols reveals that the liver disease was associated with deposition of pigment granules similar to those which we described in human subjects in South Africa. On the basis of experimental work with rats we have no hesitation in saying the hepatic lesions preceded the development of the lesions in the pancreas. Certainly, in rats, the lesion may progress and the pancreas may atrophy without any fibrosis at all, and all that remains of the pancreas are masses of fatty tissue and isolated islands without any replacement fibrosis. I think that the relationship between the liver and the pancreas in this particular lesion is of importance, especially in view of the fact reported by Dr. Baggenstoss that the presence of uremia and of renal lesions, in particular, may predispose to the lesion in the pancreas. This indicates that the entire body is reacting and the pancreas plays an important rôle in the pathologic process.

(Dr. I. Davidsohn, Chicago, Ill.) Two years ago, I observed lesions in a patient who died of ulcerative colitis. Since that time we had a few cases of the same condition and I found it also in some of the older cases with ulcerative colitis on re-examination. I do not remember that any of the cases had uremia as a significant complication.

(Dr. Mallory) I should like to make one more comment in relation to what Dr. Gillman has said. I had read his paper and was interested in the possibility of dietary deficiency. In the group of cases we studied we had a wide variety of nationalities: Italian civilians, French Moroccan, American, native Indian troops, British, and German prisoners of war, all of whom had widely different backgrounds, but the incidence of the lesion in all these different racial groups was identical.

(Dr. Gillman) May we have some information about the state of the liver in these patients?

(Dr. Baggenstoss) There were no abnormalities noted in the liver in most of the cases. We did have some cases of obstructive jaundice in which uremia developed that revealed the pancreatic lesion; but in most of the cases there were no lesions observed in the liver outside of such incidental ones as chronic passive congestion.

(Dr. Mallory) I think the liver lesions in the cases I saw were coincidental. There were instances of focal necrosis and chronic passive congestion and so on, but nothing constant or specific. In fact I was much interested to observe that we never saw the lesion in association with fatal epidemic hepatitis.

A HISTOLOGIC STUDY OF SKELETAL MUSCLE IN ACUTE ISCHEMIA.* John W. Harman (by invitation), Madison, Wis.

Abstract. Skeletal muscles of rabbits and white rats, rendered acutely ischemic for periods of from 1 to 96 hours by ligation of vessels and application of tourniquets, were studied histologically and compared with contralateral normal controls. The characteristics of normal rapidly fixed muscles are described as syncytoid structures with vague cross striations and a conspicuousness of undulant longitudinal striations. The nuclei, which are deeply basophilic, contain a fine chromatin network. With ischemia of 2 to 4 hours' duration the fibers are individualized, longitudinal striations disappear, and cross striations become a conspicuous cytologic feature. After longer periods of ischemia abnormal anisotropic disks, called "Bowman's disks" or "conchoidal plates," appear and involve the muscle fibers in

increasing numbers up to 18 hours of ischemia, at which time they are nearly ubiquitous. They represent true degenerative forms and not artefacts caused by fixation and sectioning technique. Weakness or absence of contractility precedes and accompanies the appearance of these disks, and is correlated with their presence and extent of involvement, so that they serve as an indication of nonviable fibers and represent a morphologic manifestation of cell death in skeletal muscle.

NODULAR INFLAMMATORY AND DEGENERATIVE LESIONS IN MUSCLES FROM 450 AUTOPSIES. B. J. Clawson, J. F. Noble, and (by invitation) N. H. Lufkin, Minneapolis, Minn.

Abstract. Seven muscles (the pectoral, sternomastoid, deltoid, diaphragm, intercostal, psoas, and sacrospinalis) were collected from each of 450 autopsies and studied for evidences of inflammatory and degenerative lesions.

The Inflammatory Lesions. These lesions consisted primarily of infiltration of lymphocytes and plasma cells between the muscles. They were most common in the diaphragm and intercostals and were present in various degrees in one or more muscles in 118 cases (26.2 per cent). These inflammatory lesions were most commonly found in cases of acute rheumatic endocarditis, rheumatoid arthritis, and in older persons with various diseases. The sexes were about equally involved.

The Degenerative Lesions. The degenerative lesions consisted of atrophy, sarcoplasmic changes (Zenker's degeneration and necrosis), and nuclear changes. Atrophy was most common in the diaphragm and sacrospinalis, and sarcoplasmic changes in the diaphragm. Atrophy in different degrees was noted in 191 cases (42.4 per cent) and sarcoplasmic changes in 152 cases (33.8 per cent). Nuclear changes consisted of irregularities in number, shape, and size, and of hyperchromatic staining qualities. These nuclear changes were most common in the deltoid, diaphragm, intercostal, and sacrospinalis. These changes were noted in one or more muscles in 158 cases (35.1 per cent).

One or more of the lesions (myositis, atrophy, sarcoplasmic or nuclear changes) was present in 293 of the 450 cases (65.1 per cent). On a morphologic basis it is doubtful whether any of the muscular lesions can be considered as a specific reaction to the infectious agent of either acute rheumatic or rheumatoid arthritis. These lesions may possibly be a part of the rheumatic and rheumatoid state.

Discussion

(Dr. Virgil H. Cornell, Washington, D.C.) I would like to ask if Dr. Clawson can report on the incidence of trichinosis in the diaphragm.

(Dr. Clawson) Trichinosis was commonly found. It was not included in these plates. Some of these inflammatory lesions possibly may have been on the edge of a trichinosis lesion. I did not work out the incidence. It was relatively high.

(Dr. Cornell) The high percentage in the diaphragm was what struck my eye.

READ BY TITLE

PATHOLOGY OF TRENCH FOOT. Matthew Block (by invitation), Chicago, Ill.
Abstract: Not received.

YELLOW FEVER IN THE AFRICAN. John C. Bugher and (by invitation) Richard G. Hahn, New York, N.Y.
Abstract: Not received.

THE NONESSENTIALITY OF ARGININE FOR SPERMATOGENESIS IN THE ALBINO RAT. Paul R. Cannon and (by invitation) C. Harold Steffee, Robert L. Woodridge, Laurence E. Frazier, and Norman L. Jennings, Chicago, Ill.
Abstract: Not received.

HEMOGLOBIN CRYSTALS, CASTS, AND GLOBULES IN THE RENAL TUBULES OF GUINEA-PIGS FOLLOWING CHEMICAL HEMOLYSIS.* Robert C. Dunn and (by invitation) Stewart H. Webster, Bethesda, Md.

Abstract. An experimental investigation was undertaken of the results in the kidneys of guinea-pigs following hemolysis due to exposure to stibine (SbH_3). Stibine resembles arsine (AsH_3) in its action, but hitherto has been studied very little from the biologic viewpoint. The hemoglobin deposits in the kidney (casts, crystals, and globules) were described in relation to the time elapsing after exposure to stibine. Other changes in the kidney were noted: tubular dilatation, coagulation, necrosis, cellular casts, fat, glomerular changes, and hemosiderosis. The finding and identification of hemoglobin crystals in the renal tubules we believe will be of particular interest. This study is an example of the combined application of physiology, crystallography, and biochemistry in experimental pathology.

THE ALARM REACTION AS A MEASURE OF TOXICITY OF GOITROGENIC COMPOUNDS. William E. Ehrich and (by invitation) Joseph Seifter, Philadelphia, Pa.

Abstract: Not received.

SOME APPLICATIONS OF THE FREEZING-DRYING METHOD FOR MORPHOLOGIC PROBLEMS. Isidore Gersh (by invitation), Chicago, Ill.

Abstract. The principles of the freezing-drying method of fixation make it particularly useful in certain kinds of purely morphologic problems. Several designs (by Gersh, Scott, Sjöstrand, and others) are available for easy application in investigative laboratories. Preservation of substances affected during fixation by water or by enzymes is very satisfactory. Accordingly, the method is particularly suitable for the preservation of inorganic components, glycogen, mucigen granules, edematous tissue, and thyroid colloid. Gas bubbles appearing in blood and/or tissues after decompression are also well preserved. In these instances and in others, the structure after fixation by freezing and drying may be freer of artifact, and may reproduce more closely the living appearance than after more conventional methods of fixation.

EXPERIMENTAL AND SPONTANEOUS BRUCELLOTIC OSTEOMYELITIS OF THE ANIMAL. Leo Lowbeer (by invitation), Tulsa, Okla.

Abstract. At the preceding meeting of this Association, I reported a case of human brucellotic osteomyelitis of the ilium and scapula from which *Brucella suis* was grown in pure culture. Because little is known about the cellular changes of brucellotic osteomyelitis, an attempt was made to reproduce it by inoculation of guinea-pigs with our human strain of *Br. suis*. In addition, material from spontaneous brucellotic osteomyelitis of the hog was obtained through the courtesy of the United States Department of Agriculture.

Of 7 inoculated guinea-pigs, osteomyelitis and periostitis were produced in 2. Both animals were killed 7 weeks after inoculation. One had bilateral exophthalmos caused by retrobulbar abscesses from which *Br. suis* was cultured. Both had pseudo-suppurative orchitis and epididymitis, and typical brucellotic granulomas and pseudo-abscesses in the liver and other organs, all with positive cultures. One animal showed fusiform enlargement of many ribs caused by periosteal osteophytes. There was also osteoclastic absorption of the cortex from a round-cell infiltrated periosteum with fragmentation and formation of sequestra. The cortex was also melting away from the bone marrow side through an apparently humoral process. The marrow showed necrosis and mononuclear cell infiltration. In areas of cortex destruction there was extensive formation of endosteal and periosteal osteophytes and chondrophytes.

* This article will appear in a subsequent issue of *The American Journal of Pathology.*

The other animal started limping shortly before it was sacrificed. Roentgenogram showed a destructive lesion in the epiphysis of the distal left radius. Microscopically, there was replacement of the bone marrow by partly necrotic mononuclear cell granulomas and destruction of cancellous bone; osteoclastic absorption of bony spicules took place in the neighborhood. Multiple similar areas were found in other bones.

Three cases of spontaneous brucellotic spondylitis and one of osteomyelitis of the radius and ulna of hogs with positive cultures of *Br. suis* also were studied. The lesions all resembled those presented by Feldman and Olsen in 1933.

In conclusion, brucellotic osteomyelitis, spontaneous as well as experimental, causes mononuclear cell infiltration and necrosis of the bone marrow, occasionally with the formation of pseudo-abscesses, early osteoclastic absorption of bone through caries sicca and humoral destruction of bone, and very extensive and early peripheral production of new bone. This leads to the formation of a central cavity filled with necrotic, often calcified granulation tissue and sequestra, and surrounded by a fibrotic capsule and new formed bone. The relatively few roentgenologic and clinical studies of human brucellotic osteomyelitis indicate that the process in man is similar to that in the animal.

CYTOLOGY AND INCIDENCE OF INTRANUCLEAR INCLUSIONS IN THE ISLANDS OF LANGERHANS OF THE CHICKEN.* Alfred M. Lucas (by invitation), East Lansing, Mich.

Abstract: Not received.

A SURVEY OF 52 CASES OF ACUTE NECROSIS OF THE LIVER. J. S. McCartney, Minneapolis, Minn.

Abstract: Not received.

RELATIONSHIP OF DEGREE AND DURATION OF HYPERTHERMIA TO THE NATURE OF THE CUTANEOUS INJURY. A. R. Moritz and (by invitation) F. C. Henriques, Jr., Boston, Mass.

Abstract. Predictable reactions of porcine and human skin to thermal exposures were obtained by appropriate regulation of the surface temperature and the duration of the hyperthermic episode. The occurrence of latent and morphologically unrecognizable thermal injury was disclosed by the cumulative effects of repeated subthreshold exposures. The sequence of events during exposure at temperatures of different intensity was studied with particular attention to the occurrence of a type of tissue denaturation that rendered it resistant to lysis and organization.

METASTATIC CALCIFICATION PRODUCED IN DOGS BY HYPERVITAMINOSIS D AND HALIPHAGIA.* R. M. Mulligan, Denver, Colo.

Abstract. Seven dogs receiving vitamin D (as ertron) and sal hepatica (containing the chloride, sulfate, phosphate, and bicarbonate salts of sodium) and 5 given ertron alone showed weight loss, inappetence, hypodypsia, and apathy, which 2 dogs on sal hepatica alone and 2 control dogs did not exhibit. The dogs in each of the 4 groups displayed characteristic stools. All dogs receiving ertron revealed varying calcification in the left atrium, aorta, lungs, stomach, and kidneys, and various degrees of atrophy of the parathyroid glands, prostate, testes, and lymphoid and fat tissues; some of them showed hypoplasia and serous fat atrophy of the bone marrow.

HEMOLYTIC ANEMIA AND DUODENAL ATRESIA IN ONE OF TWINS IN A CASE OF HETEROSPECIFIC PREGNANCY WITH ISO-IMMUNIZATION OF THE MOTHER. Silik H. Polayes, Brooklyn, N.Y.

Abstract: Not received.

* This article will appear in a subsequent issue of *The American Journal of Pathology.*

DIASTASIS AND DIASTATIC PERFORATION OF THE GASTROINTESTINAL TRACT. A CLIN-
ICAL, PATHOLOGIC, AND EXPERIMENTAL STUDY. Jacob M. Ravid, New York,
N.Y.

Abstract. Diastasis and diastatic perforation of the gastrointestinal tract is a
clinicopathologic entity, first described by Heschel in 1880, which has not received
due recognition by either clinicians or pathologists. It is a distention, partial tearing,
or perforation of a nondiseased bowel, which takes place as a secondary complica-
tion in the course of an intrinsic or extrinsic stenosing disease of the bowel situated
distally to the site of distention or perforation. It is most common in cancer of
the colon, but may occur also with congenital disease of the gastrointestinal tract,
volvulus, and various other intrinsic or extrinsic obstructive diseases of the intes-
tines. The clinical picture is that of chronic intestinal obstruction with sudden
transition to "an acute abdomen." The competence or incompetence of Bauhin's
valve plays an important rôle in this syndrome. Experimental work done in this
connection furnishes interesting data with regard to the "tensile" strength of the
various portions of the gastrointestinal tract, which can be correlated with the
anatomic findings in the observed clinical cases.

THE INFECTIVITY OF SYPHILITIC MOUSE ORGANS. Paul D. Rosahn and (by invi-
tation) Boris Gueft, New Britain, Conn.

Abstract. Groups of mice were inoculated by different routes with rabbit
chancre emulsions. At variable intervals following inoculation different mouse
organs were subinoculated into rabbits which were observed for the development
of darkfield-positive lesions. Spleen and lymph nodes were regularly infectious at
10, 20, 30, 45, and 90 days following mouse inoculation. The frequency of successful
transfers to rabbits, when infected mouse skin was employed, increased pro-
gressively to almost 100 per cent at 90 days after inoculation. Mouse brain, how-
ever, was noninfectious at 10 and 20 days, and at 90 days after inoculation was
capable of producing lesions in rabbits in only about half of the trials. The findings
varied somewhat, depending on the method of inoculation.

THE CONTAGIOUSNESS OF COCCIDIOIDOMYCOSIS. AN EXPERIMENTAL STUDY. Sol
Roy Rosenthal, Chicago, Ill.

Abstract. The spherules of *Coccidioides immitis* remain viable under certain
conditions and do not produce hyphae and chlamydospores in exudates from human
sources for at least 110 days (experiments still in progress). By instilling sputum
or exudates from human or animal sources into the bronchi of guinea-pigs and
propelling them by air pressure into the finer ramifications of the bronchioles and
alveoli, it is possible to produce coccidioidomycosis infections in 100 per cent of
the animals. The lesions localize for the most part in the upper portions of the lung,
are single or multiple, have a lymph node component, and do not generalize; thus
simulating the human infection. These experiments show that spherules (or sporan-
gia) can be infective through the respiratory route from man to animal, and from
animal to animal. It is concluded that until proved otherwise, active human
cases of primary progressive coccidioidomycosis should be considered contagious.

GASTRIC CANCER: A COMPARISON OF THE GROSS AND MICROSCOPIC DIFFERENCES
BETWEEN SHORT TERM AND FIVE-YEAR SURVIVORS AFTER GASTRECTOMY. Paul
E. Steiner and (by invitation) S. N. Maimon, Walter L. Palmer, and Joseph
B. Kirsner, Chicago, Ill.

Abstract. Thirty patients who survived for 5 years or more after gastric resec-
tion for cancer were compared with 30 who died of local recurrence or metastases
within 1 year after a similar operation. Attempts were made to find points of
prognostic significance between the two groups by studying the clinical data,
and the gross and microscopic appearances of the tumors. Differences in the clinical

data were not striking, but the cancers in the 5-year survivors were grossly more sharply circumscribed as indicated by a greater percentage of the lower types by the Borrmann method. Three histologic differences were found between the two groups. In order of importance they were: (a) sharp circumscription with growth of the tumor through the gastric wall *en bloc,* seen in most of the 5-year but in few of the short-term survivors; (b) retrogressive changes consisting of atrophy, nuclear pyknosis, and degeneration, found in a few 5-year survivors (these changes resemble those present in some prostatic carcinomas following orchiectomy); (c) two special histologic types of cancer: one an undifferentiated, medullary small-celled carcinoma with leukocyte-rich stroma; the other an undifferentiated round cell sarcoma or carcinoma. Presence of metastases at the time of operation was not incompatible with 5-year survival. Biologically, the long-term survivors were not a homogeneous group. Survival for 5 years was explained by complete removal of tumor cells in some cases and by slow rate of local recurrence or metastasis in others. The biologic resistance to rapid growth was anatomically manifest by the three histologic features mentioned, which can be used to advantage in forecasting prognosis in gastric cancer.

LOCALIZATION OF THE KIDNEY DAMAGE INDUCED BY *dl*-SERINE IN THE RAT. PHOSPHATASE ACTIVITY, INFLUENCE OF AGE, SEX, TIME, AND DOSE. PROTECTIVE ACTION OF VARIOUS AMINO ACIDS AND SOME OTHER COMPOUNDS. Max Wachstein, Middletown, N.Y.

Abstract. The findings of Fishman, Artom, and Morehead concerning the nephrotoxic action of *dl*-serine in rats were fully confirmed. Not only male animals weighing 100 gm. but also weanlings and adult rats are susceptible. In female rats, likewise, *dl*-serine exerts a nephrotoxic action, although less regularly.

The minimal injurious dose for male rats weighing 100 gm. on a synthetic diet poor in the B vitamins and deficient in protein was found to be 10 mg., while 20 to 30 mg. led in most animals to extensive necrosis. As early as 30 minutes after intravenous injection of *dl*-serine, distinct kidney lesions were observed in some instances. The renal damage was found to be localized in the terminal portions of the proximal convoluted tubules. After the introduction of *dl*-serine, phosphatase activity was still found 24 hours later in the cells of necrotic tubules.

The severe necrotizing nephrosis induced by *dl*-serine can be favorably influenced by several amino acids and related compounds. *dl*-Methionine and glutathione exert a considerable protective influence. This is probably not due to SH-groups, since cysteine and thioglycolic acid have only little, and 2·3-dithiopropanol (BAL) has no, beneficial effect. *dl-a*-Alanine, glycine, *dl*-threonine, glycolic acid, butyric, and pyruvic acids have a very considerable protective influence. *l* (+) Histidine monohydrochloride and lactic acid afford appreciable, *l* (+) arginine monohydrochloride and *dl*-valine moderate, protection. Some influence was seen from *l* (+) glutamic acid, while glucose, sodium acetate, and sodium chloride were without any effect. It is assumed that the beneficial effect of the various amino acids and other substances against the nephrotoxic action of *dl*-serine is due to their competitive suppression of tubular reabsorption of the injurious *d*-isomer.

HEPATOMA REMOVED WITH, TO DATE, A FIVE MONTH CLINICAL RECOVERY. J. W. Williams and (by invitation) C. C. Woods, Bay Pines, Fla.

Abstract. A 64-year-old white male began to have dizzy spells and peristaltic pain in the upper abdomen, worse at night, aggravated by food, and followed by nausea 4 months ago. At operation, a single tumor mass, replacing the left lobe of the liver and the size of a cocoanut, was removed surgically. On section the tumor was brownish red and of scrambled egg consistency. Microscopically, it showed areas ranging in appearance from leiomyosarcoma, and angiosarcoma to hepatoma. The patient gained weight, and now, 5 months after operation, is clinically well.

STUDIES OF THERMAL INJURY
III. The Pathology and Pathogenesis of Cutaneous Burns
An Experimental Study *

A. R. Moritz, M.D.

(From the Department of Legal Medicine, Harvard Medical School, Boston, Mass.)

In Study II of this series,[1] measurements of the reciprocal relationships of time and surface temperature with respect to the capacity of thermal exposures to destroy the epidermis of man and pig were reported. This report concerns the pathogenesis and pathologic characteristics of cutaneous burns in relation to the duration and intensity of thermal exposure and to their susceptibility to organization, repair, and healing.

Source of Material

The material used for pathologic study was derived from several sources as indicated in Table I. Many of the hot water burns of both human and porcine skin were made in Study II of this series to establish the reciprocal relationship of time, temperature, and epithelial destruction. The method of their production has been fully described. Since the majority of the lesions described in Study II were not excised until they had been under clinical observation for days or weeks, many duplicate exposures were made and excised in order to observe the sequence of microscopic changes that took place between the incurrence of various types of injury and their repair. To acquire this material, approximately 60 additional hot water exposures of pigs' skin were made and examined microscopically after recovery periods ranging from a few seconds to several weeks. Additional porcine material was derived from a series of burns made by exposure to hot air at temperatures varying between 80° and 900° C.[2]

There were two series of human burns, one comprised of 33 experimentally produced lesions which were studied clinically but were not excised for microscopic examination, and the other of skin specimens obtained at post-mortem examination of victims of accidental conflagrations.

Sections of tissue for microscopic examination were cut from specimens that had been fixed·in Zenker-formol or 4 per cent formaldehyde solution. Phloxine and methylene blue stains were made routinely

TABLE I

Sources and Kinds of Material Used for Study of the Pathogenesis of Cutaneous Burns

Subject	Source of heat	Range of exposure		Range of recovery period
		Temperature	Duration	
Pig	Water	44° to 100°C.	0.5 sec. to 7.5 hrs.	1 min. to 4 wks.
Pig	Air	80° to 900°C.	0.5 sec. to 45 min.	1 min. to 3 days
Man*	Water	44° to 60°C.	3 sec. to 6 hrs.	1 min. to 4 wks.
Man	Air	?	?	Less than 1 hr.

* Lesions not excised for microscopic study.

and were augmented by sections stained with hematoxylin and eosin or by Pollak's modification of Masson's trichrome method. Many sections were stained by the Feulgen technic.

General Consideration of the Quantitative and Qualitative Effects of Heat on the Skin

A cutaneous injury caused by hyperthermia may be characterized quantitatively according to the depth to which the tissue has been destroyed, or qualitatively according to the nature of the changes that have occurred. The characterization in Tables II and III in Study II of hyperthermic episodes as sub-threshold, threshold, and supra-threshold refers to their quantitative capacities for injury production, the determining factor being whether the exposure in question is insufficient, just sufficient, or more than sufficient to cause transepidermal necrosis.

Thus, any exposure that failed to cause complete destruction of the epidermis was designated as sub-threshold and any reaction short of transepidermal necrosis was one of the first degree. A second degree reaction was one caused by the shortest exposure at any given temperature, or the lowest temperature at any given time that resulted in full-thickness destruction of the epidermis. Although it was not possible to destroy the entire thickness of the epidermis without some damage to the underlying connective tissue, dermal necrosis was a

relatively insignificant feature of a threshold exposure. A third degree reaction was one caused by an exposure that was supra-threshold in respect to time or temperature and was accordingly one in which a significant degree of dermal necrosis usually accompanied the destruction of the epidermis.

When account is taken of the potential variations in the intensity and duration of the different thermal exposures that are capable of producing burns of similar severity, it becomes apparent why thermal lesions may be qualitatively dissimilar even though their ultimate effect in terms of the amount of tissue destroyed is the same. This fact is more readily appreciated by reference to Text-Figures 1 and 2. The
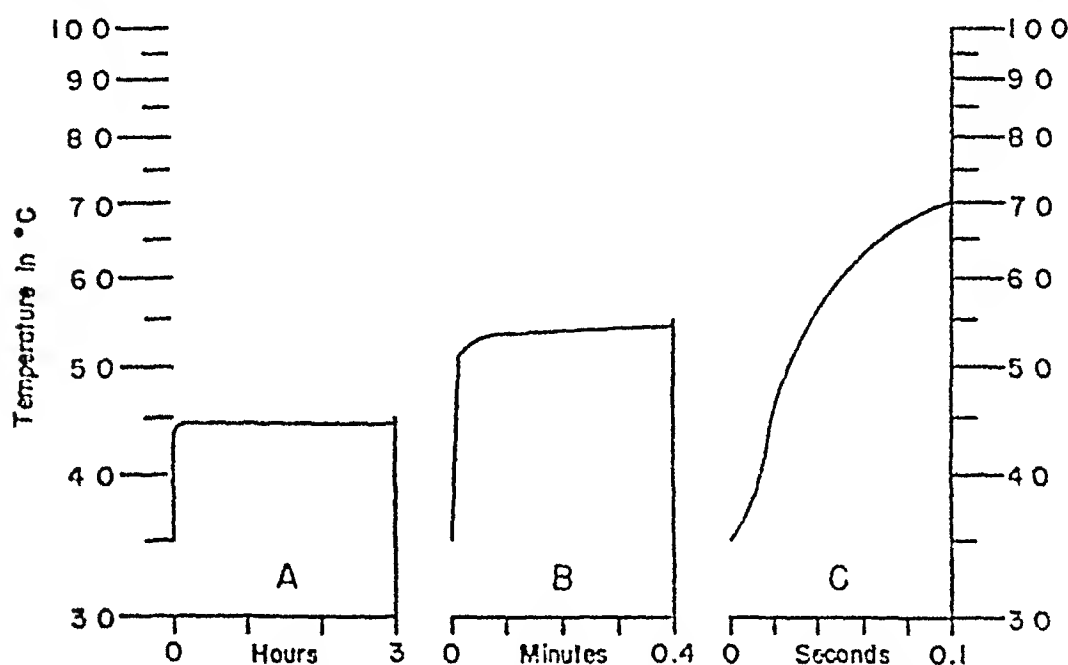
Text-Figure 1. Curves depicting the change in temperature that would occur at the interface between dermis and epidermis during surface exposures of 45° (A), 55° (B), and 100° C. (C). Each of these was a threshold exposure in that 3 hours, 0.4 minutes, and 0.1 second, respectively, are estimated to be the shortest time at the indicated temperature that would cause transepidermal necrosis. (Curves derived from data reported by Henriques and Moritz.[3])

critical temperature, so far as the ultimate fate of the epidermis is concerned, is that attained at the interface between epidermis and dermis rather than that of the surface. In Text-Figure 1 are shown the estimated changes * in temperature that would occur at the basal cell level during the course of thermal exposures at three different surface temperatures if each was terminated at a time calculated to be just adequate to destroy the epidermis. In Text-Figure 2 are shown the temperatures that would prevail at different depths below the sur-

* Calculations based on data presented in Study I of this series.[2]

face of each at the moment that the duration of the exposure was just sufficient to cause irreversible injury of the entire. thickness of the epidermis.

In each instance, the effects would be quantitatively similar, in that irreversible cellular injury would extend to, but not far beyond, the basal cell layer. That qualitative differences in the resulting reactions might exist despite their quantitative similarity can be inferred from the fact that in the exposure shown in Text-Figure 1-A, the epidermis
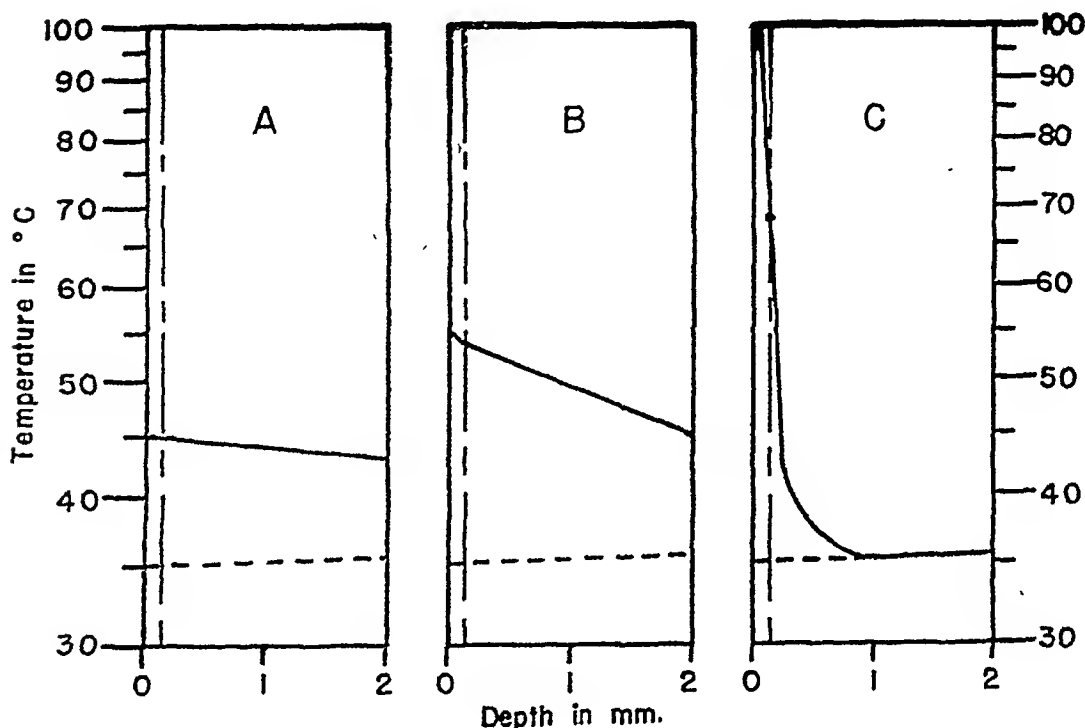
Text-Figure 2. Curves depicting the temperature gradient through the skin that would exist at the conclusion of three types of thermal exposure at the termination of an exposure just sufficient to cause epidermal necrosis. The solid line traversing each chart from left to right depicts the temperature gradient from the surface to a depth of 2 mm. (dermis-fat interface). The transverse broken line depicts the pre-exposure gradient through the same thickness of skin. The vertical broken line indicates the approximate depth (circa 100 μ) of the dermis-epidermis interface. In A, the surface of the skin had been maintained at 45°C. for 3 hours. At the end of this time the temperature at a depth of 2 mm. had been raised from 36° to 44° C. In B, the surface of the skin had been maintained at a temperature of 55°C. for 0.4 minutes. At the end of this time the temperature at a depth of 2 mm. had been raised from 36° to 47°C. In C, the surface of the skin had been maintained at 100°C. for 0.1 second. At the end of this time there had been no change in the temperature at a depth of 2 mm. (Curves derived from data reported by Henriques and Moritz.[3])

was destroyed by a 3-hour episode of hyperthermia, the intensity of which at no time rose above 44.8° C. at the basal cell level. Approximately the same amount of irreversible change would be sustained as the result of the exposure depicted in 1-C. In the latter

instance, the epidermis would be destroyed in approximately 0.1 second by an episode of hyperthermia in which the temperature at the basal cell level rose sharply and briefly to 70° C. The exposure depicted in 1-B falls about midway between these extremes. Although the total amount of irreversible injury is about the same in each, it is to be expected that the three lesions produced by these exposures would differ qualitatively.

An additional reason for the occurrence of qualitative differences in quantitatively similar reactions to thermal exposures of different intensity is shown in Text-Figure 2, in which are depicted the calculated transcutaneous thermal gradients to a depth of 2 mm. that would exist at the moment of completion of the same three episodes of hyperthermia that are illustrated in Text-Figure 1. In each instance, irreversible thermal injury would extend to, but not appreciably beyond, the basal cell layer. In the exposure depicted in 2-A, the temperature of the dermis to a depth of about 2 mm. would be elevated above the normal level for at least 2 hours. In the exposure depicted in 2-C, the transcutaneous thermal gradient was so steep that the resulting temperature changes in the dermis were exceedingly brief and superficial. It is apparent why the epithelial cells would be destroyed in 2-C with relatively little disturbance of the dermis, whereas in 2-A the same or even a lesser degree of epidermal injury would be accompanied by a severe and persistent vascular disturbance.

FIRST DEGREE REACTIONS
Hyperemia, Edema, and Cyanosis

Dilatation of the superficial vessels sufficient to cause visible reddening of the skin usually followed, and, in the case of exposures of low intensity, characteristically preceded the occurrence of recognizable damage to the epidermis. An exception to this generalization was the absence of vascular reaction in animals suffering circulatory failure. In such there was frequently a depression of vasomotor irritability so profound that injurious episodes of hyperthermia of either high or low intensity failed to elicit vascular reactions despite the occurrence of extensive epidermal injury. Another circumstance in which thermal damage of the epidermis was sustained with little or no vascular reaction was when an exposure to intense heat was so brief that, although the surface was burned, there was little or no rise in dermal temperature.

In the case of flash (circa 0.5 second) exposures to flame temperatures it was possible to carbonize superficial shreds of the stratum corneum without causing sufficient rise in sub-surface temperature to

damage the basal layer of epithelial cells or to cause a perceptible vascular reaction. The initial transepidermal thermal gradients established by such exposures were so sharp that the presence of a thin film of moisture on the surface of the skin was sufficient to make the difference between reaction and no reaction in the case of near-threshold exposures.

Attention has already been called to the fact that the duration of an episode of hyperthermia of low intensity must be greatly prolonged if it is to produce an injury quantitatively comparable to one resulting from an exposure of high intensity. Since the dermal blood vessels are far more responsive to temperature changes than are the epithelial cells, it can be understood why severe and persistent vascular reactions were often elicited by protracted episodes of hyperthermia of low intensity that failed to harm the epidermis (Text-Fig. 1).

There was considerably greater variation among human subjects than among pigs in respect to the vascular reactions to cutaneous hyperthermia. The variability of dermal vascular reactions in human subjects was so great and the number of reactions studied in this investigation so few that little could be inferred as to the extent to which animal data can be applied to man in this respect. The impression was gained that the thermal stimulus necessary to cause visible erythema in most human subjects was substantially lower than that required to elicit erythema in the pig. In man the change in skin color was usually more intense and of longer duration than after an identical exposure in the pig.

That an active circulation of blood was maintained through the dilated capillaries of an evanescently erythematous skin was indicated in part by the pink or red color of the surface and in part by the fact that the surface temperature * during such a reaction was characteristically between 0.5° and 1.5° C. higher than that of the adjacent skin.

An evanescent erythematous reaction to heat could not, as a rule, be recognized in sections prepared for microscopic examination. Vessels, the seat of physiologic dilatation, usually contracted during or immediately after excision, and it was difficult or impossible to distinguish a sample of physiologically hyperemic skin from one that was normal or even ischemic.

When cutaneous hyperthermia was prolonged to between 40 and 60 per cent of the minimum time required for the production of transepidermal necrosis in either man or pig, it characteristically resulted in a severe and pathologic vascular reaction with edema and cyanosis,

---

* Determined by means of the fine wire thermocouple described in Study VIII.[4]

and one which persisted for days rather than minutes or hours. That the flow of blood through the dilated capillaries was slowed was indicated by the blue or purple color of the surface in contrast to the pink or red color caused by the more evanescent active hyperemia. The surface temperature of such a lesion during the first few hours was frequently found to be from 0.5° to 2° C. below that of the adjacent normal skin. That the reaction was pathologic rather than physiologic was indicated also by the fact that in both man and pig it was almost invariably accompanied by cutaneous edema. Within the first hour after the onset of a vascular injury of this grade, the water content of the dermis was increased by as much as 100 per cent.

Microscopic examination of reactions of this type at varying periods after exposure in the pig confirmed the clinical observation in both pig and man that thermal exposures of relatively low intensity may result in severe and protracted disturbances of the dermal blood vessels without causing irreversible damage to the overlying epithelial cells. The capillary loops of the dermal papillae became dilated, elongated, and filled with closely packed masses of erythrocytes. Separation of collagen fibers by edema fluid was obvious and perivascular mantles of extravasated erythrocytes were often seen. The escape and extravascular deterioration of erythrocytes were often sufficient to result in brown discoloration of the target area for as long as 1 week, due to the formation of hemosiderin. Extravascular fibrin was not encountered nor did the collagen fibrils appear to be swollen. Between 12 and 24 hours after such an injury was sustained, occasional polymorphonuclear leukocytes were found in the edema fluid. Neither thrombosis nor visible alteration in the vascular endothelium was seen despite the fact that superficial vessels were filled by static, sausage-like agglomerates of red blood cells.

Reversible Impairment of Epidermal Anchorage.

During most, and possibly all, injurious episodes of cutaneous hyperthermia in which the temperature of the epidermis was maintained for a sufficient time at 49° C. or higher, there was a brief interval subjacent to the threshold for transepidermal necrosis during which there was reversible impairment of the adhesion of epidermis to dermis.

The attainment of this degree of injury was recognized by the ease with which the epidermis could be dislodged by friction. If the exposure was discontinued before further injury was sustained and if the loosened epidermis was not dislodged by trauma or vesication, the change was potentially reversible in the case of the pig. After

12 or 18 hours the original firm anchorage of the epidermis was usually regained. Unless the exposure had been excessive, such injuries subsided without evidence of cell death.

When skin altered in this manner was protected against mechanical artifact, there was no microscopic evidence in either the epithelium or the underlying dermis by which impairment of the epidermal anchorage could be recognized. However, when sufficient friction was applied to the temporarily insecure porcine epidermis to cause its detachment, microscopic examination revealed a fringe of uprooted or fractured tonofibrils protruding from the lower ends of the detached basal epithelial cells. The protruding fibrils appeared to have been pulled out of their anchorage in the superficial dermal feltwork of collagen fibers. It was not determined whether the essential change responsible for such epidermal instability was a deterioration of the extracellular extensions of the tonofibrils, which predisposed them to rupture, or a softening of the dermal collagen in which they were embedded. The latter was considered the more plausible explanation of the phenomenon. In man it is doubtful that the tonofibrils of the epidermal cells have much if anything to do with the attachment of epidermis to dermis. In human skin, the epidermis appeared to be cemented to, rather than rooted in, the dermal collagen.

It has already been indicated that when porcine skin sustained this type of cutaneous burn, recovery sometimes took place within 24 hours without death of cells, providing the damaged area was protected against mechanical disturbance during that period when its anchorage to the dermis was insecure.

Too few appropriate specimens of human burns were available for microscopic examination to permit conclusions regarding the threshold at which, or the frequency with which, this particular type of first degree thermal injury occurs in man. The opinion was gained from clinical observations of human burns that thermal exposures insufficient to cause primary epidermal necrosis may result in a temporary impairment in the adhesion between epidermis and dermis. If such a temporarily insecure layer of epidermis is detached by friction or vesication, it will undoubtedly die if it remains dislodged. It is entirely possible that the phenomenon of vesication results, in some instances, in secondary destruction of human epithelial cells that would otherwise survive. If this be true, and if the thermal exposure has been insufficient to cause primary transepidermal necrosis, the immediate institution of pressure to prevent epidermal displacement by vesication should predispose to early and uncomplicated healing of what might otherwise become an open lesion.

Irreversible Thermal Injury of Epidermal Cells

Material for microscopic study was available from almost every conceivable kind, grade, and stage of thermal injury of the skin of the pig. Although a wide range of experimental thermal injuries of human skin was studied clinically, most of the burns that were available for microscopic examination were obtained from autopsies. Thus, there was no direct information regarding the intensity or duration of the thermal exposures that were responsible for the burns of human skin that were studied microscopically. The impression was gained however, that apart from the phenomenon of vesication, the cytologic changes induced by heat in the epidermis of man were similar, if not identical, to those observed in the pig (Figs. 1 to 6). Attention has already been directed to the fact that the time-temperature threshold for the destruction of epidermis is almost identical in human and porcine skin.

The first microscopic evidence of irreversible thermal injury of the epidermis consisted of what appeared to be a change in the distribution of water and solids within the nuclei of the cells of the intermediate zone. As the nuclei swelled, their chromatin granules coalesced to form compact crescentic masses immediately beneath and attached to one side of the nuclear membranes. When the swollen nucleus ruptured, the peripherally distributed chromatin contracted to form a dense and irregularly shaped central mass which remained separated from the surrounding cytoplasm by clear fluid. This fluid, whether extruded into the nuclear lacuna or contained within the distended nuclear membrane, was faintly basophilic and sometimes contained a few, fine, Feulgen-positive fragments of chromatin.

Pyknosis of nuclei was by no means pathognomonic of thermal injury. Spontaneous nuclear pyknosis was sometimes seen in the upper zone of unheated epidermis and was caused by injuries other than heat.

In the case of sub-threshold exposures sufficient to injure the upper layers of epidermal cells, but insufficient to cause transepidermal necrosis, the above-described types of nuclear change were frequently focal and difficult to distinguish from qualitatively similar changes in control material. Even though it could be plausibly assumed that all of the cells at a given level were exposed to the same degree of hyperthermia, it was not uncommon to find groups of cells with normal appearing nuclei interspersed among those with nuclei that showed advanced degenerative change (Fig. 2). The reason for this apparent difference in the susceptibility of cells in the same layer to heat was not apparent.

When the thermal exposure was of sufficient intensity or duration to cause irreversible cellular injury, nuclear changes of the kinds de-

scribed in the foregoing paragraphs were usually apparent immediately after the conclusion of the episode of hyperthermia. This was not invariably the case, however, and after certain exposures at relatively low temperature (under 47° C.) a post-exposure interval of between 6 and 12 hours was sometimes required for the development of recognizable nuclear alterations. Moreover, when the exposure was of sufficient severity to cause pseudovesication in the pig or true vesication in man, many of the nuclei which were apparently undamaged at the conclusion of the exposure disintegrated during the next 24 hours.

When the episode of hyperthermia was such as to cause visible alterations in nuclear structure, there was inhibition of mitotic division throughout the entire area of exposure for many hours. There was no evidence derived from the microscopic study of sub-threshold exposures to indicate that hyperthermia predisposed to acceleration of mitotic activity. The impression was gained that nuclear swelling with coalescence of chromatin granules constituted evidence of an irreversible cellular change and invariably led to premature death and desquamation of the altered cells.

In the pig, the irreversibly damaged epidermal cells were gradually desquamated over a period of 1 week to 10 days in the form of thin brown scales.

Alterations in the appearance of nuclei in the upper and intermediate layers of epithelium were thought to provide the earliest morphologic evidence of primary thermal injury of the epidermis and were frequently encountered without perceptible damage to the cells of the basal layer. Characteristically, the earliest change in the basal cell layer caused by hyperthermia was cytoplasmic rather than nuclear. The injured basal cells swelled and their cytoplasm became vacuolated and increasingly acidophilic. The vacuolization appeared to be due in part to imbibition of fluid and in part to redistribution of water and solids within the cells.

The fluid contained within the cytoplasmic vacuoles was clear, non-sudanophilic, and sometimes contained a delicate mesh of granular amphophilic débris. With severe injury there was widespread rupture and disintegration of the lower ends of the basal cells.

SECOND DEGREE REACTIONS

Transepidermal Necrosis

The time-temperature characteristics of exposures just sufficient to cause transepidermal necrosis in both man and pig are indicated in Text-Figure 4 of Study II. In man, it could usually be determined whether or not a thermal exposure had destroyed the epidermis by

the occurrence or nonoccurrence of vesication within the first 24 hours. To recognize with certainty during the first day or two after a near-threshold exposure whether or not porcine epidermis had been destroyed it was necessary to excise the skin and examine it microscopically. When the area of injury was 7 mm. in diameter and when the duration and intensity of the exposure was at or not far above the threshold required for transepidermal necrosis, the time usually required for complete healing was between 5 and 10 days in the pig and between 1 and 2 weeks in man.

In the pig, microscopic evidence that an exposure had been sufficient to cause transepidermal necrosis was provided by the changes that had occurred at the basal cell level. With the disintegration of the cytoplasm of the proximal or lower ends of the injured basal cells, there was at first, focal, and later, extensive, spontaneous detachment of the epidermis from the dermis. In the pig, the amount of fluid that collected beneath the loosened epidermis was never sufficient to produce true vesication.

With still more severe hyperthermia, the cytoplasmic disintegration of the basal cells was followed by nuclear changes similar to those seen in the more superficially located cells. When the epidermal detachment was incomplete, stretching and attenuation of the remaining bridging cells and their nuclei were often seen. Such attenuated masses of chromatin were often stretched to two or three times the original length of the entire cell.

In the event that the surface temperature of the epidermis was brought rapidly to a level of 55° C. or higher, and maintained at such a level long enough to produce a third degree burn, transepidermal coagulation usually masked any nuclear swelling that may have occurred. In such an event, neither the cytoplasm nor the nuclei of the epithelial cells appeared swollen (Fig. 7). On microscopic examination, both appeared shrunken, the former being intensely acidophilic and the latter small and homogenously basophilic.

Vesication

Attention has already been called to the fact that a common effect of heat on the skin of both man and pig is impairment of the attachment of the epidermis (Figs. 5 and 6) to the dermis, and the opinion was expressed that this may have been due either to a change in the physical state of the superficial dermal collagen or to disruption of the basal layer of epithelial cells. A common collateral effect of cutaneous hyperthermia, and one that was apparently essential to true vesication, was an outpouring of fluid from the dermal capillaries.

When a thermal exposure of human skin was sufficient to impair the attachment of the epidermis, the amount of edema fluid that collected between it and the dermis was usually sufficient to elevate and stretch the entire layer of dead, dying, and living cells to form a vesicle. Although vesication of human skin was usually an almost immediate response to a thermal exposure of sufficient severity to cause primary epidermal injury, there were several circumstances in which it was either delayed or inhibited.

Delayed vesication was most frequently seen after exposures of long duration at low temperatures or after flash exposures at high temperatures. In both circumstances it seemed likely that the delay was due to the fact that the vascular damage was relatively mild, and that hours rather than minutes were required for enough fluid to collect beneath the damaged epidermis to form a vesicle. Also vesication was delayed or prevented when the injury was so overwhelming that the dermis and its superficial capillaries were almost immediately coagulated. With such thermal injury, the level at which edema developed was too deep to result in vesication (Fig. 8). Thus, in man, the nonoccurrence of vesication after a thermal exposure sufficient to cause severe injury of the epidermis may mean that the dermal hyperthermia was either inadequate to result in edema or that it was so overwhelming that the superficial capillaries were almost immediately occluded.

In no instance was true vesication of porcine skin observed. This was true despite the fact that many of the injuries met a prerequisite to vesicle formation, namely, sufficient impairment of the adhesion between epidermis and dermis to permit easy mechanical detachment of the former (Fig. 5). Failure of the pig to vesicate appeared to be due to the fact that the amount of edema fluid that penetrated the surface of the dermis was never sufficient to elevate the epidermis. In the absence of evidence to the contrary, a tenable explanation for nonvesication in the pig was that an episode of hyperthermia that was sufficient to impair the attachment of the epidermis to the dermis characteristically altered either the superficial feltwork of dermal collagen fibers or the walls of the capillaries contained by it, in such a way that they became virtually impermeable to edema fluid.

The nature, or for that matter, the existence, of this theoretical alteration in the permeability of the collagen or the capillary walls was not disclosed by microscopic examination. When the severity of an exposure was considerably in excess of that required to destroy the epidermis, swelling of the superficial dermal collagen and occlusion of its capillaries could be recognized. There was a wide range of exposures between the threshold for epidermal necrosis and that for recognizable

swelling of collagen or occlusion of capillaries in which the microscopic examination of the pig's skin disclosed no explanation for failure of porcine skin to vesicate.

THIRD DEGREE REACTIONS

The more a thermal exposure exceeded the threshold required for destruction of the epidermis, either in respect to temperature or time, the deeper the injury and the longer the recovery period necessary for repair and regeneration. In both pig and man, several weeks represented the minimum healing time if a significant degree of dermal injury had been sustained.

Further Changes in the Epidermis

In the case of the pig, prolonged exposure at a relatively low surface temperature (under 55° C.) caused relatively little additional change in the microscopic appearance of the epidermis. In the higher range of surface temperatures, a significant prolongation of the rate of duration of exposure beyond the time necessary to destroy the epidermis modified the quality of the superficial changes both in human and porcine skin. In man, vesication was permanently inhibited and in both man and pig the loosened epidermis became reattached to the damaged dermis (Figs. 7 and 8). Early shrinkage of both the cytoplasm and the nuclei of the damaged cells occurred before there was opportunity for the development of the retrogressive changes observed in first and second degree reactions. The higher the temperature, the shorter the time required to cause transepidermal coagulation. With exposures to superheated air, desiccation was superimposed on the effects of heat, and soon after the temperature rose above 300° C., carbonization of the dry tissue began to take place.

Red and Pale Burns

The surface color of third degree burns ranged from pale gray through red, purple, and brown to black, depending on certain attributes of the exposures responsible for their production.

A black or carbonized surface resulted from exposures at temperatures in excess of 300° C. (Fig. 9). The precise temperature at which carbonization began was not determined.

A red, purple, or brown surface, due to the presence of blood in the superficial layer of the skin, resulted from exposures in which the dermal temperature was raised slowly enough to permit advanced engorgement of the superficial capillary plexus before the occurrence of coagulation.

A gray or ischemic surface indicated that the upper portion of the dermis had undergone thermal coagulation before the superficial capillaries had become fully engorged.

The reciprocal relationships of time and temperature as they relate to the visibility of hemoglobin beneath the surface of a third degree thermal reaction are shown in Table II of Study II. It was found that at atmospheric pressure and at surface temperatures of 65° C. or lower, burns appeared superficially hyperemic regardless of the duration of exposure. When a 70° C. surface exposure was interrupted at the end of 2 seconds, the lesion remained red, but if it were prolonged

TABLE II

Increase in Hemoglobin Iron in Skin and Subcutaneous Tissue following Thermal Injury

Condition of skin	Weight of iron per sq. cm. of sample	Estimated weight of blood per sq cm. of sample
	(μg.)	(mg)
Normal	2.4	5.5
	4.0	9.0
Moderate hyperemia	5.6	13
	12	26
Severe hyperemia	22	50
	24	55
	16	35
	16	35
	15	34

to 3 minutes, the zone of reactive hyperemia became overlaid by so thick a layer of coagulated tissue that it was no longer visible. Above 70° C. all exposures of 1 second or longer coagulated the superficial plexus of dermal capillaries so rapidly that most or all of the blood contained in them was displaced to a level too deep to be seen from the surface.

POOLING OF BLOOD IN HYPEREMIC BURNS

A qualitative impression of the pooling of blood in the dilated cutaneous vessels after an injurious episode of hyperthermia was derived from the photomicrographs shown in Text-Figure 2 of Study II. In order to learn something of the actual amount of blood that was present in such lesions, samples of both normal and hyperemic skin were excised for chemical examination. Samples of skin and subcutaneous tissue having an area of 25 sq. cm. and extending to the deep fascia were taken from the lateral thoracic area of each of 9 pigs and their iron content was determined.[5] Two of the samples represented normal skin and the other 7 were from areas of hyperemic burning.

It is apparent from the results of the experiments shown in Table II that a relatively large proportion of the total circulating blood of an animal may be pooled in the skin and subcutaneous tissue as a result of thermal injury. Calculations based on the amount of recoverable iron per unit of surface area of burned skin in relation to the body weight indicated that as much as 30 per cent of the erythrocytes of an animal suffering from generalized cutaneous hyperemia could be accounted for in the skin and subcutaneous fat.

THE EFFECT OF COMPRESSIVE HYPERTHERMIA ON THE COLOR OF A BURN

In Study II, attention has been called to the fact that pressure on the skin surface during exposure to heat does not increase the vulnerability of the epidermis to thermal injury except as it may improve the contact between a hot solid and the skin surface. It was found, however, that compression of the skin was capable of modifying the superficial color of the burn even though there was no increase in the severity of the lesion. To determine the circumstances in which compression of the skin during an episode of hyperthermia may modify the color of the burn, the experiments on pigs summarized in Table III

TABLE III
The Effect of Pressure upon the External Appearance of Burns

Temperature of surface	Duration of hyperthermia	Pressure on skin	External appearance of burn 24 hours after exposure	
			Ischemic	Hyperemic
(°C.) 70	(seconds) 5 5	(mm. Hg) 0 120	+	+
65	30 30 60 1200	0 120 120 0	+	+ + +
60	60 60 120 300	0 120 120 0	+	+ + +
55	1800 1800	0 120		+ +

were undertaken. In some, hot water was applied at atmospheric pressure, and in others it was applied with a compressive force of 120 mm. of Hg.

The results of these exposures indicated that the color of burns resulting from surface temperatures lower than 55° C. was not affected by pressure, but that an increase in pressure during exposures at

surface temperatures of 60° C. or higher determined whether the surface of the resulting burn would be ischemic or hyperemic. Thus, an exposure at atmospheric pressure at 60° C. produced a red burn even though it was extended for as long as 5 minutes. With increase in pressure, a 2-minute exposure at the same temperature resulted in a pale burn and yet the depth to which the tissue had been destroyed in the latter was less than that to which it had been destroyed in the former. At 70° C., a 5-second exposure at atmospheric pressure resulted in a red burn, but with an additional pressure of 120 mm. of Hg the resulting burn appeared ischemic.

Microscopic examination of these lesions provided evidence that the color of a burn was not a reliable criterion by which to judge its depth. After hyperthermic episodes of comparable duration and at the same surface pressure, a red surface color usually indicated that the lesion was less severe than one having a gray surface. Without knowledge of time, temperature, or surface pressure during the period of exposure, it is not possible to estimate the relative severity of burns on a basis of surface color.

OTHER EFFECTS OF HEAT ON THE DERMIS

After edema and pericapillary extravasation of erythrocytes, the earliest recognizable extravascular alteration of the dermis was swelling of collagen fibers. This occurred first in the most superficial layer where, in the case of porcine skin, the projecting tonofibrils of the basal epithelial cells were imbedded in the collagen of the subjacent connective tissue.

As the intensity and duration of the hyperthermia increased, the corium tended to lose its fibrillar character and became a thin lamella of homogeneous acidophilic material as though the individual fibers had been converted to a gel. With increasing exposure the swelling of collagen became apparent at greater and greater depths in the underlying connective tissue (Fig. 8). Expansion of collagen bundles tended to collapse and obliterate the loose perivascular areolar tissue. Contracted blood vessels appeared thick-walled and empty. Visible edema receded in advance of this type of alteration as though the fluid were imbibed or displaced by the denatured collagen. Not until 24 or 48 hours had elapsed was it possible by microscopic examination to recognize the line of demarcation between reversible and irreversible dermal injury.

From the intact blood vessels of the deeper and relatively uninjured tissues leukocytes migrated upward through the perivascular interstices and into the zone of denatured collagen. A frontier was eventu-

ally established between the tissue capable of recovery and that des-
tined to be sequestered in the form of a desiccated crust. The deeper
the lesion the longer the time required for the stabilization of such a
frontier. The transition between the obviously necrotic tissue of the
upper dermis and the least disturbed tissue of the deepest portion
of the zone of hyperthermia was a gradual one.

Exudation of leukocytes occurred within a few hours, and within 24
hours usually served to delineate the zone within which the plane of
irreversible injury would eventually become stabilized. Within 2 or
3 days, fibroblasts and new capillaries began to push up toward the
surface in the interfascicular interstices of the denatured collagen
(Fig. 10). The least affected connective tissue at the base of the reac-
tion zone recovered quickly and without apparent loss of fixed tissue
cells. The fate of the more severely injured collagen varied according
to the extent to which it had been denatured. Thermal denaturation
of collagen at temperature levels under 55° C. (Fig. 11) did not appear
to result in the kind of coagulative change that made the collagen
resistant to subsequent autolysis and organization. Collagenous de-
naturation at temperatures over 55° C. often resulted in an irreversible
type of coagulation which resisted lysis and eventually led to seques-
tration en masse. Thus, deep and severe burns resulting from surface
exposures lower than 55° C. were likely to remain soft and red and
the necrotic tissue was susceptible to organization. Deep burns result-
ing from higher temperatures were characteristically firm and pale
and there was sequestration but not organization of the necrotic tissue.
Between these two extremes the dead and damaged connective tissue
was infiltrated by leukocytes and penetrated by granulation tissue,
and its necrotic elements were gradually resorbed and replaced by
new connective tissue.

During the time required to establish the level of irreversible injury,
tentative tongue-like masses of new epithelial cells grew out from the
margins of the lesion and from the viable roots of partially destroyed
hair follicles as though they were seeking a sufficiently well stabilized
layer of connective tissue to provide support and nutrition. Repeated
crops of such new epithelial cells extended over or into the granulation
tissue and failed to survive, for reasons not disclosed by microscopic
examination.

Summary

The transfer of heat to the skin at a rate sufficiently great to raise
the sub-surface temperature to an appreciably higher level than that
which is normal for the organism leads to a series of local reactive and
alterative changes, the severity of which bears a direct relationship

to the degree and duration of the temperature rise. The nature of the change that occurs at any given depth below the surface of the exposed skin is determined in part by the intensity and duration of the temperature rise at that level and in part by the nature of the affected tissue.

The mildest form of epidermal injury produced by hyperthermia is latent in the sense that it is not associated with recognizable alteration in cell structure. Such injuries are reversible and the time required for their reversal increases in direct relation to the time required for their production.

The earliest visible evidence of thermal injury of the epidermis is a redistribution of chromatin within, and swelling of, the nuclei, first in the intermediate and later in the deepest layer of epithelial cells. Further injury results in swelling and disintegration of the cytoplasm of the basal cells and pyknosis of nuclei throughout the entire thickness of the epidermis. As the result of alterations in the basal cells or in the cement substance that binds them to the dermis, hyperthermia may result first in a reversible and subsequently in an irreversible impairment of the attachment between epidermis and dermis. If the temperature rise within the epidermis does not exceed a level of approximately 55° C., further primary thermal alterations of the epithelial cells either do not occur or are so slow in development as to be of negligible significance.

When the exposure is such as to result in a progressive rise in subsurface temperature, the next step in the succession of alterative changes is the occurrence of transepidermal coagulation. When the epidermal temperature is raised rapidly to a level higher than 55° C., coagulative changes may mask both nuclear swelling and cytoplasmic disintegration. Further increases in the temperature of the epidermis result in progressive desiccation and eventually in carbonization.

The earliest visible alterative change in the dermis in response to hyperthermia is constriction of the superficial blood vessels. Ordinarily, this is followed almost immediately by vasodilatation. When the rise in dermal temperature is sufficiently rapid and high, the superficial vessels become permanently fixed in their initial reactive state of constriction and vasodilatation occurs only at deeper levels where the temperature rise has been less extreme.

Thermal vasodilatation characteristically leads to increased vascular permeability and edema formation. The escape of edema fluid at any particular sub-surface level requires, first, that the vascular injury at that level has been sufficiently severe to result in increased mural

permeability and, second, that it has not resulted in cessation of blood flow through the damaged vessels.

Although thermal exposures of appropriate intensity and duration impair the attachment of epidermis to dermis and increase the permeability of the superficial capillary plexus of the dermis in both pig and man, it is only in the latter that a sufficient amount of edema fluid collects beneath the epidermis to cause true vesication.

Absence of vesication in human skin that has been exposed to heat may indicate that the rise in dermal temperature was insufficient to cause increased vascular permeability, that insufficient time has elapsed for the collection of enough fluid beneath the epidermis to cause appreciable elevation, that the hyperthermia was so extreme as to have caused cessation in blood flow through the superficial capillaries, or that cutaneous ischemia and loss of vasomotor irritability were antecedent to the exposure to excessive heat.

As in the case of the epidermis, coagulative changes occur in the dermis when its temperature is raised to, and maintained for a sufficient period at, a level higher than 55° C. Further increases in dermal temperature may result in desiccation and carbonization.

It was found that the superficial appearance of a burn is not a reliable criterion by which to judge the depth to which the tissue may have been irreversibly injured. The surface of a relatively shallow burn may show coagulation and even carbonization if the exposure has been intense and brief, whereas a thermal exposure of insufficient intensity to coagulate even the most superficial portions of the skin may, nevertheless, cause deep destruction if sufficiently prolonged.

Although the quantitative results of a short exposure of high intensity may be similar to those of a long exposure at low intensity, there are likely to be significant qualitative differences between such injuries. Hyperthermia of high intensity results in a coagulative type of necrosis in which the dead tissue does not undergo autolysis, resists organization, and is usually disposed of by sequestration. Hyperthermia of low intensity results in a noncoagulative type of necrosis in which the dead tissue undergoes autolysis and is readily susceptible to organization.

Biopsy of a burned area within 24 hours should provide useful information pertaining to the depth and nature of injury. Recognition by microscopic examination of dermal coagulation would indicate eventual sequestration of the tissue so affected. Recognition of a zone of irreversible injury would indicate the need for debridement before attempting to graft new epidermis on the surface.

REFERENCES

1. Moritz, A. R., and Henriques, F. C., Jr. Studies of thermal injury. II. The relative ·importance of time and surface temperature in the causation of cutaneous burns. *Am. J. Path.*, 1947, 23, 695–720.

2. Moritz, A. R., Henriques, F. C., Jr., Dutra, F. R., and Weisiger, J. R. Studies of thermal injury. IV. Exploration of casualty-producing attributes of conflagrations. The local and systemic effects of generalized cutaneous exposure to excessive circumambient (air) and circumradiant heat of varying duration and intensity, *Arch. Path.*, 1947, 43, 466–488.

3. Henriques, F. C., Jr., and Moritz, A. R. Studies of thermal injury. I. The conduction of heat to and through the skin and the temperatures attained therein. A theoretical and an experimental investigation. *Am. J. Path.*, 1947, 23, 531–549.

4. Henriques, F. C., Jr. Studies of thermal injury. VIII. Automatic recording caloric applicator and skin tissue and skin surface thermocouples. *Rev. Sci. Instruments.* (In press.)

5. Wong, S. Y. Colorimetric determination of iron and hemoglobin in blood. II. *J. Biol. Chem.*, 1928, 77, 409–412.

DESCRIPTION OF PLATES

PLATE 136

FIGS. 1 and 2. Photomicrographs of severe first degree burns of porcine (Fig. 1) and human (Fig. 2) skin showing the degenerative changes in epidermis. In Figure 1 there is generalized pyknosis of nuclei, and none of the epidermal cells included in the picture would have recovered. In Figure 2 the changes are focal rather than general and most of the altered nuclei are swollen and show peripheral displacement of the chromatin. This type of nuclear change precedes that shown in Figure 1. Both specimens were excised within 1 hour after the injury was ·sustained. In both instances the epidermal attachment to the dermis was insecure and the lesion shown in Figure 2 probably would have gone on to vesication in the normal course of events. X 400.

FIGS. 3 and 4. Photomicrographs of early second degree burns of porcine (Fig. 3) and human (Fig. 4) skin showing early spontaneous detachment of the epidermis from the dermis. Vacuolar cytoplasmic disintegration of the basal cell layer has been added to nuclear changes similar to those shown in Figures 1 and 2. In Figure 3 the tonofibrils that were uprooted from their anchorage in the dermis can be seen projecting from the detached basal cells. X 400.

2

4

1

3

Thermal Injury

Moritz
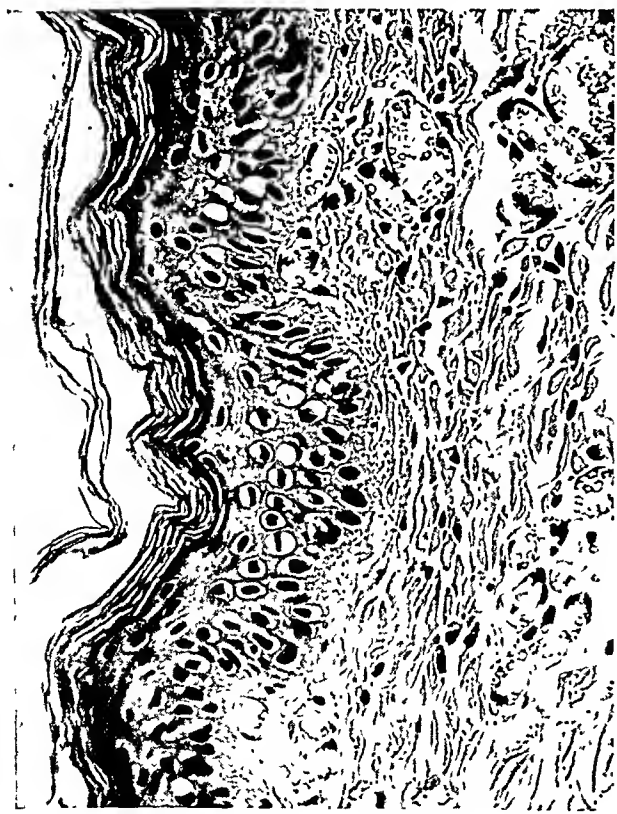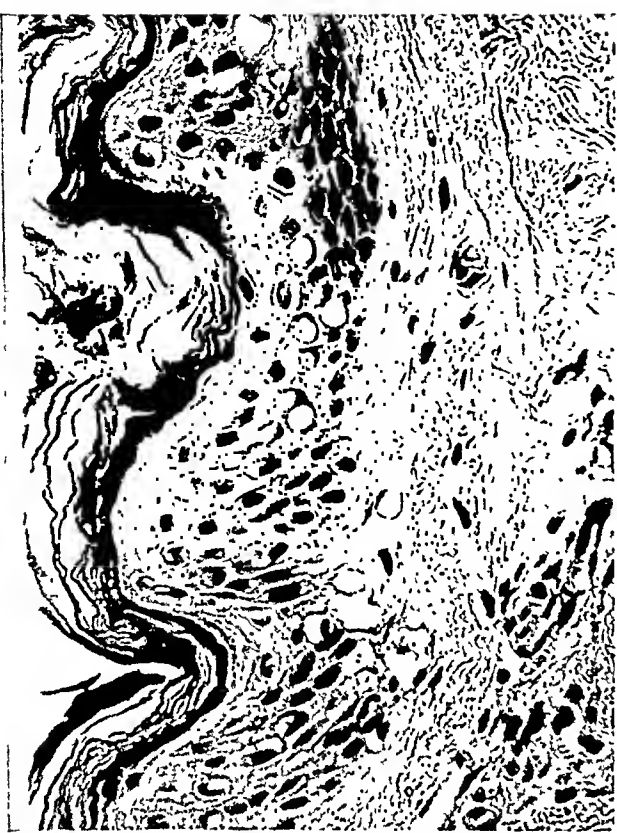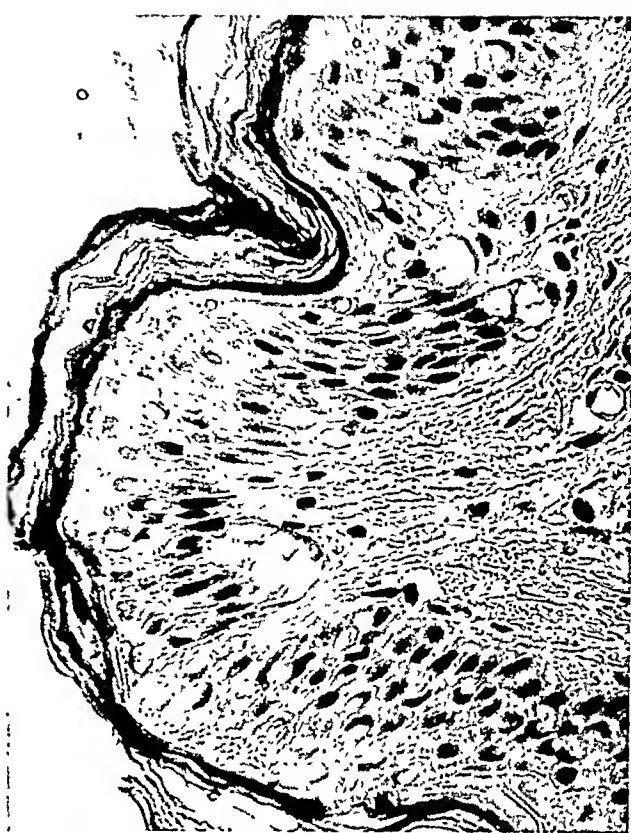
FIGS. 5 and 6. Photomicrographs of a pseudovesicle of porcine skin (Fig. 5) and an early true vesicle of human skin (Fig. 6). In each, transepidermal necrosis is complete. In the case of porcine skin the detached epidermis would have remained *in situ* as a flaccid membrane. In the case of human skin the detached epidermis would have been elevated by the collection of edema fluid between it and the dermis. × 400.

FIG. 7. Third degree thermal injury of porcine skin showing coagulation of epidermis and dermis 24 hours after injury. Burn produced by maintaining surface of skin at 65° C. for 2 minutes. The bundles of denatured dermal collagen appeared swollen and homogeneous, and had become increasingly basophilic. Thermal reactions of this type were encountered only when the surface temperature had been brought to, and maintained at, a level of 55° C. or higher. Coagulative changes of this type render the necrotic tissue resistant to lysis and organization. × 400.
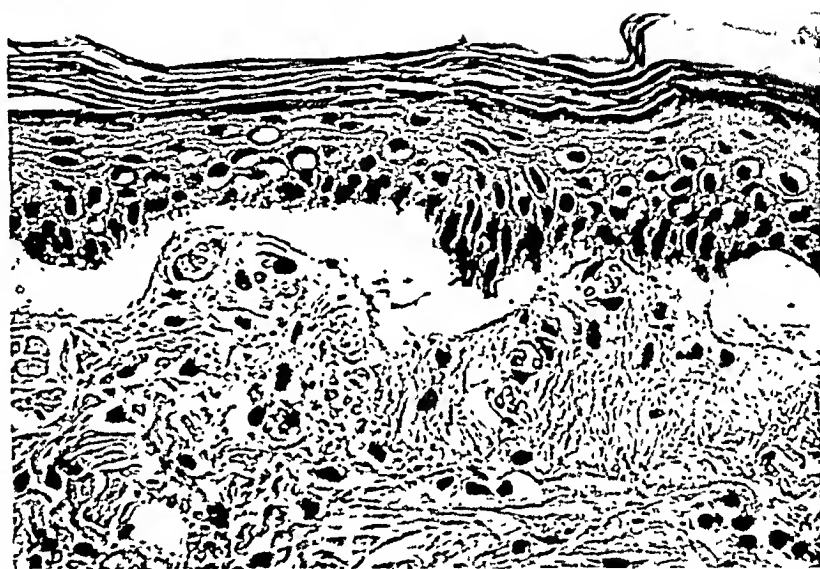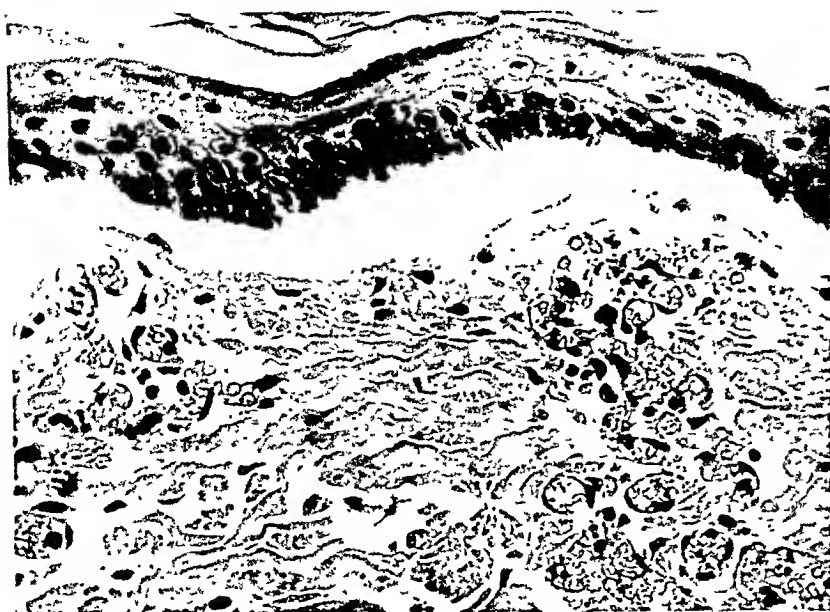
PLATE 138

FIG. 8. Transcutaneous coagulation resulting in a deep ischemic lesion, caused by a 5-minute exposure to circumambient (air) temperature of 200° C. × 85.

FIG. 9. Carbonization of surface of skin with desiccation and intense basophilia of coagulated dermis, caused by an exposure of 2.5 minutes to a circumambient (air) temperature in excess of 400° C. × 85.

PLATE 138

8

9

Moritz
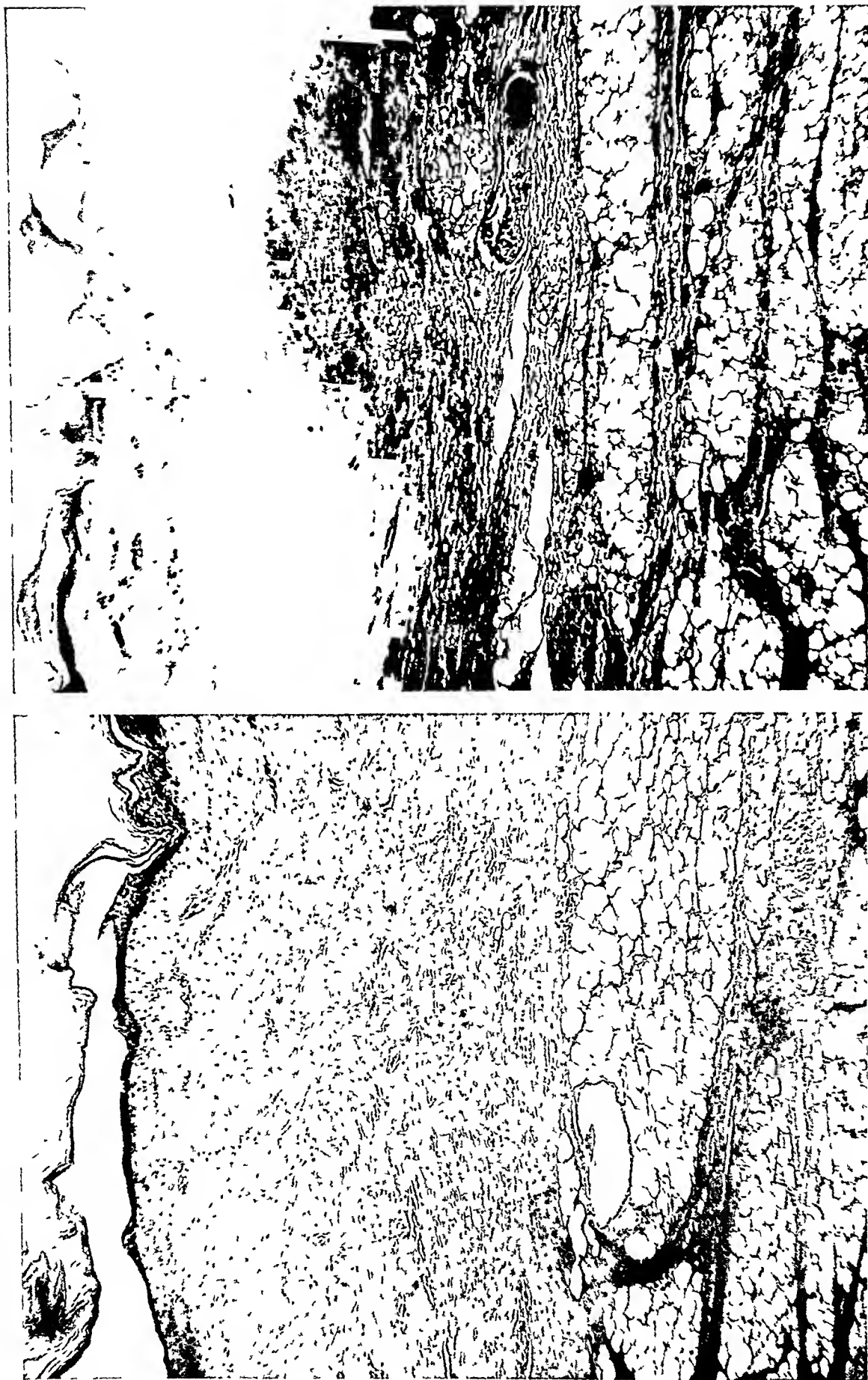
Thermal Injury

PLATE 139

FIG. 10. Photomicrograph of a third degree thermal reaction in porcine skin 72 hours after injury. Exudative cells have migrated into the interstices between the bundles of coagulated collagen. The precise level at which this injury will be stabilized is not yet apparent. Healing will be slow because of the resistance of the denatured collagen to autolysis and organization. $\times$ 400.

FIG. 11. A 24-hour-old third degree burn of porcine skin in which the necrotic dermal tissue was not coagulated, was already densely infiltrated by exudative cells, and was readily susceptible to organization and repair. Burn produced by maintaining the surface of the skin at 53° C. for 5 minutes; for comparison with burn shown in Figure 7 in which coagulation of dermal collagen had rendered it resistant to lysis and organization. $\times$ 400.
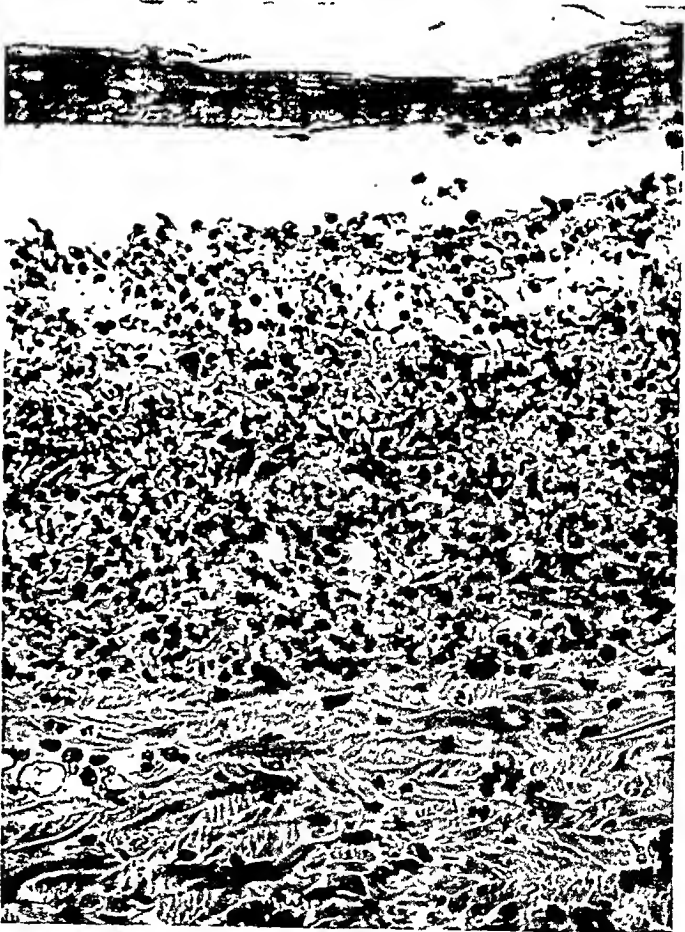
GRANULOMA, A CHARACTERISTIC "QUALITATIVE" CHANGE IN FOCAL ANAPHYLACTIC INFLAMMATION [*]

J. W. GODDARD, M.D.

(From the Department of Pathology, Long Island Hospital, Boston·Harbor, Boston, Mass.)

I. INTRODUCTION

Nature of the Anaphylactic Reaction. The "Quantitative" Hypothesis

The effects of Arthus' "Repeated Injections of Horse Serum into the Rabbit," [1] presented as a note to the Société de Biologie in June, 1903, became soon afterwards, under the name of "Arthus' phenomenon," the starting point of innumerable immunologic, physiologic, biochemical, and clinical·researches on anaphylaxis, hypersensitiveness, and allergy. His second note, with Maurice Breton, on "Cutaneous Lesions Induced by Horse Serum Injections into Rabbits Anaphylactized by and for this Serum" [2] was presented to the same Society a few months later (November, 1903). This note, dealing with the histopathologic aspects of local shock, did not share the·fate and importance of the first article, which dealt with its physiologic implication. The reason is to be found in the fact that the histopathologic picture of the focal anaphylactic response, as described therein, is not at variance with a "common" inflammatory reaction, except for some heightening of the process.

The assumption that focal anaphylaxis was pathologically similar to a common type of inflammation needed verification. Controlled researches were not performed, however, until 1914. Between 1914 and 1923, Rössle,[11,12] and, under his instigation, Fröhlich,[3] and Gerlach [4,5] attacked the problem anew. The experimental method consisted either of a reproduction of the classical Arthus' phenomenon (on rabbits, and other animals; Gerlach) or of appropriate modifications. The latter comprised (1) watching the initial·stages of the anaphylactic response and their development on the transparent mesentery of a living and sensitized frog, with substitution of the shock antigen for an inflammation-inducing agent (Fröhlich); (2) studying the local lesion induced by subcutaneous injection of avian erythrocytes into specifically sensitized guinea-pigs, the local shock being elicited when the serum of the guinea-pigs reached a high titer of anti-avian hemolysins (Rössle); (3) observing, through "abdominal windows," the gross peritoneal changes in specifically sensitized animals when the shock antigen is introduced into the peritoneal cavity (Rössle).

These controlled researches, which brought forth some additional

data (presence of eosinophilia, for example) failed nevertheless to invalidate the initial findings of Arthus and Breton.[2] It is repeatedly maintained by the authors cited that the local anaphylactic reaction is not "qualitatively" different from any inflammatory response, the changes being simply and solely "quantitative." These quantitative changes concern the suddenness of the reaction; the immediately ensuing and more or less sustained blood stasis; the tremendous edema, which may or may not be hemorrhagic; the abundant eosinophilia, and the necrosis of the vessels walls. The process is heightened, accelerated, and shortened, and "stormy" in its unfolding. It fixes and limits the injurious shock antigen to its portal of entry, and therefore protects the organism as a whole, sometimes at the expense of local tissue death, through the interplay of capillary contraction, walling off by exudation, and the neutralizing and diluting effects of the edema.

Evidence Against the "Quantitative" Hypothesis

The accepted opinion, outlined above, that local anaphylaxis is only a "quantitative" variant of inflammation does not appear tenable, for the following reasons:

(a) Inflammation and anaphylaxis are two distinct phenomena. Inflammation follows a wide range of inciting agents and in final analysis is due to the injury or death of a cell or group of cells. Anaphylaxis, on the other hand, is highly specific, in that it depends upon an active or passive sensitization, a latency period, and, at least in part, on some form of antigen-antibody reaction. Anaphylaxis could be only a special case of inflammation, and as such its histologic picture should show more than "quantitative" changes.

(b) The anaphylactic response is the result of a primary antigen-antibody coupling, and occurs not in the blood stream or tissue fluids, but on or in cells which are able to remove or fix the circulating antibody. The anaphylactic state, moreover, is associated with the presence of a "fixed" antibody, and, conversely, with the absence of a freely circulating antibody. Immunologists have long since discarded the "humoral" or "anaphylatoxin" hypothesis, which supposes the formation of an antigen-antibody complex, and the adsorption by the latter of a stabilizing serum constituent with subsequent digestion of serum proteins or release of a toxic compound. Instead, immunologists now rally to the "cellular" hypothesis. It is beyond the aim of the present article to submit the experimental evidence which is so strongly in favor of the view that the anaphylactic response is cellular in nature and origin. However, we are still ignorant of whether the

phenomenon occurs at the surface or in the interior of cells, the types of cells concerned, and the histologic modalities of the phenomenon.

If, therefore, during the anaphylactic shock, the cells alone are operative, it seems fair to assume that a responsive cell may be found which will show by histologically analyzable signs its dominant rôle in the phenomenon. One may venture to say that its reactivity must be specific and "qualitatively" different from a common inflammatory response.

Rôle of the Histiocytes

Experimental evidence points to only two types of cells which seem to respond during the anaphylactic shock: the smooth muscle cell and the reticulo-endothelial cell. The reactivity of the smooth muscle cells explains, among other experimental and clinical data, the Schultz-Dale phenomenon and that of the "effector organ" (which varies from species to species, according to the richness of smooth muscle cells in these organs). However, in focal anaphylactic reactions of the Arthus type, the smooth muscle cells cannot be considered because of their scarcity in the subcutaneous tissue, where they are found only in the vessel walls. The reticulo-endothelial cells, on the other hand, are ubiquitous and can be found in the active mesenchyme of the subcutaneous tissue, not only as primitive mesenchymal cells, endothelial and perithelial cells, but also as histiocytes and monocytes.

The rôle of the histiocytes and their derivatives in anaphylaxis is still a debatable question (Jungeblut [6]), although experiments indicate a strict relationship between functional responsiveness of the reticulo-endothelial system and the anaphylactic shock. India-ink blockade attenuates and in cases inhibits shock when the specific antigen is injected. Similarly, the duplication of Arthus' initial experiment fails to induce an Arthus' phenomenon when the shock antigen is injected into a previously "blocked" area (experiment of the so-called local blockade, Klinge [7]).

Another argument in favor of the reactivity of the histiocytes is adduced by the behavior of the capillaries, which, with their potentially histiocytic endothelial and perithelial cells, are an integral part of the reticulo-endothelial system. Some of their reactions are only physiologic and not demonstrable microscopically, such as their functional insufficiency (the so-called irritability state of Doerr). In more advanced instances the damage becomes morphologically demonstrable as in the necrosis of the vessels, which is a prominent feature of the local anaphylactic reaction.

Finally, it has been shown by Rich,[8-10] both clinicopathologically

and experimentally, that in serum sickness the lesion is essentially a monocytic adventitial and perivascular infiltration.

If, then, the histiocytes and derivative cells, and the cells making up the capillaries are physiologically reactive during the anaphylactic shock, an investigation of the histologic behavior of the histiocytes as a whole, and of the endothelial and perithelial cells in particular, seems indicated.

With the above-mentioned considerations as a starting point, experiments have been performed to investigate the reactivity of the histiocytes and capillaries in focal anaphylactic response. Other experiments, concerning the behavior of the histiocytes in local anaphylactic reactions in animals blocked with trypan blue, and the histopathology of sensitized areas after injection with histamine, are in progress.

II. Experimental Data

All experiments were performed on healthy male and female guinea-pigs of 250 to 600 gm. average weight, kept on the usual diet of "kitchen greens" (cabbage, carrots, beets, etc.). Egg white was used as sensitizing and shock antigen. The sensitizing antigen was administered both intraperitoneally (for general sensitization) and subcutaneously (for focal sensitization). On occasion the sensitization of the focal area was reinforced by a subsequent subcutaneous injection given 24 hours after the first sensitizing injection. The site chosen for the focal injections was the mid-abdominal wall. Each subsequent injection, whether with the shock antigen or with the nonspecific antigen, was made in the same area as far as it was possible to do so, taking as a guide either the previous needle puncture mark or the grossly visible and palpable tumefaction.

In the identically sensitized control animals the shock antigen was replaced with nonspecific antigen (horse serum; in some experiments, human and rabbit serum). In one of the experimental groups, both the shock and nonspecific antigens were applied in dry form, as dressings, on a denuded area of the sensitized region.

The experiments are given in tabular form (Table I). The scope of each experimental group is summarized in the heading preceding its description in the text.

Most of the animals showed varying physiologic responses when the shock antigen was injected; they ranged from restlessness to polypnea, which was marked but of short duration. Only one guinea-pig showed the characteristic hair bristling over the head and neck ("lion's mane" sign), and recovered quickly.

The histologic technic was identical for all animals, and consisted

in Zenker's fixation, paraffin embedding, and staining with Mallory's phosphotungstic acid hematoxylin and with Mallory's phloxine and methylene blue. Occasionally Masson's trichrome stain and Foot's silver impregnation of reticulin fibers were used.

Group I. Inflammatory Changes Induced in Sensitized Animals by Subcutaneous Injection of Specific (or Shock) and Nonspecific (Control) Antigens

A. Shock Antigen. There were four findings of note in the animals receiving specific antigen.

(1) Presence of a cavity, heavily outlined by coarse and wavy fibrinous fibers. This cavity is believed to have contained the resorbed specific antigen.

(2) Presence of monocytes, histiocytes, and related cells in streamers or in loosely packed and ill-delimited islets. They were either scattered throughout or bordered the cavity which had been emptied of antigen.

(3) Presence of endothelial and perithelial hypertrophy and hyperplasia, of intraluminal blocking by monocytic forms, and evidence of endothelial and perithelial metamorphosis into monocytes, histiocytes, and related cells. The endothelial cells revealed their prospective potencies not only in the capillaries but also in precapillary arterioles.

(4) Presence of perivascular (Figs. 1 and 4), perineural (Fig. 3), and peri-adipose and intra-adipose granulomata (Fig. 2).

The remainder of the changes were consistent with a residual inflammation heightened by a recently superimposed acute inflammation. Edema, contiguous with the empty cavity, was tremendous and dissociated the stroma. The fragmented collagenous fibers showed swelling and occasional hyalinization, and were blurred or glassy. There was scattered erythrocytic diapedesis with no signs of a scavenger reaction. Neutrophilic polymorphonuclear leukocytes were scant, while eosinophils were numerous and lymphocytes rather rare. Venules occasionally were plugged with fibrin thrombi. Growth stimulation of fibroblasts was early.

B. Controls. In the animals which received nonspecific antigen the general picture was that of a residual inflammation with superimposed recent, mild, acute inflammatory changes and with no evidence of granulomata or of endothelial and perithelial reactivity. Coarsely granular coagula, presumably incompletely resorbed nonspecific antigen, were demonstrable. Edema was scant to moderate. Eosinophils were rare. In the scattered small areas of hemorrhages, groups of red cells were surrounded by macrophages.

TABLE I

Tabular Summary of Protocols of Experiments

Experiment	Group I		Group II	
	A	B (control)	A	B (control)
Number of guinea pigs	4	2	3	2
Day 0	1 ml. egg white intraperitoneally; 1 ml. egg white subcutaneously (sensitization)		3 ml. egg white intraperitoneally; 4 ml. egg white subcutaneously (sensitization)	
1	1 ml. egg white subcutaneously (reinforcement of local sensitization)			
10				
11			Linear incision, 1 cm. long on sensitized area. Wound dressed twice daily with:	
			Powdered egg albumin (shock antigen)	Powdered horse serum (nonspecific antigen)
12			Dressings as above	
13	2 ml. egg white subcutaneously (shock antigen)	2 ml. horse serum subcutaneously (nonspecific antigen)	Dressings as above	
14	2 ml. egg white subcutaneously (shock antigen)	2 ml. horse serum subcutaneously (nonspecific antigen)	Dressings as above	
15	2 ml. egg white subcutaneously (shock antigen)	2 ml. horse serum subcutaneously (nonspecific antigen)	Dressings as above	
16	2 ml. egg white subcutaneously (shock antigen)	2 ml. horse serum subcutaneously (nonspecific antigen)	Dressings as above	
17	*	*	*	*
20				
29				
31				
Granulomata	Present	Absent	Present	Absent

* Animals sacrificed.

Group III				Group IV			
A	B	C	D (control)	A	B	C	D
1	1	1	1	3	3	3	3
3 ml. egg white intraperitoneally; 4 ml. egg white subcutaneously (sensitization)				2 ml. egg white intraperitoneally; 3 ml. egg white subcutaneously (sensitization)			
4 ml. egg white subcutaneously (reinforcement of local sensitization)				3 ml. egg white subcutaneously (reinforcement of local sensitization)			
				4 ml. egg white subcutaneously (shock antigen)	3 ml. horse serum subcutaneously (nonspecific antigen) (re-sensitizer)	3 ml. human serum subcutaneously (nonspecific antigen)	
4 ml. egg white (shock antigen) subcutaneously—diluted			4 ml. egg white subcutaneously—undiluted				
1:500	1:100	1:10					
1:500	1:100	1:10	4 ml. egg white subcutaneously—undiluted				
1:500	1:100	1:10	4 ml. egg white subcutaneously—undiluted				
1:500	1:100	1:10	4 ml. egg white subcutaneously—undiluted				
1:500	1:100	1:10	4 ml. egg white subcutaneously—undiluted				
*	*	*	*				
				4 ml. egg white subcutaneously (shock antigen)	2 ml. horse serum subcutaneously (nonspecific antigen becomes shock antigen)	2 ml. horse serum subcutaneously (nonspecific antigen)	
				4 ml. egg white subcutaneously	3.5 ml. horse serum subcutaneously	3 ml. rabbit serum subcutaneously (nonspecific antigen)	3 ml. egg white subcutaneously (shock antigen)
				*	*	*	*
Present	Present	Present	Present	Present	Present	Absent	Present

* Animals sacrificed.

Group II. Wound Healing Type of Inflammation. Topical Application of Shock and Nonspecific Antigens, in Dried Form

A. Shock Antigen. In the animals given specific antigen the denuded area was covered by fibrin-entangled ghosts of polymorphonuclear leukocytes, which rested upon an irregularly patterned network of capillary sprouts. These sprouts had hyperchromic endothelial and perithelial nuclei and hypertrophic cytoplasmic bodies. The new capillaries centered or surrounded granulomata. Some granulomata showed early intratubercular or peritubercular fibrosis (Fig. 6). Beneath was a dense histiocytic as well as fibroblastic granulation tissue, which housed swollen and blurred collagenous bundles. It harbored a rich network of reactive, newly laid capillaries and also granulomata.

Granulomata were more numerous and larger in surrounding areas, outside, but near the wound, under the intact skin. Here evolutive and involutive changes were intermingled. In these granulomata the histiocytes might show fibroblastic metamorphosis or congregate into syncytia or giant cells, or be interspersed with plasmacytes. Encapsulation of solitary granulomata and conglomeration of granulomata, absent in the areas uncovered by epithelium, were well represented in the para-focal areas of the granulation tissue, which were protected by the skin.

B. Controls. In the animals given nonspecific antigen there was no evidence of granulomata, of focal accumulations of histiocytes or related cells, or of endothelial or perithelial cell reactivity. The network of capillary sprouts was radially patterned. The granulation tissue appeared somewhat looser and contained fewer histiocytes than that of the experimental animals. Connective tissue fibers showed no alterative changes.

Group III. Shock Antigen in Varying Dilutions

Granulomata were found in all of the experimental animals.

There seemed to be no necessary relationship between the strength of the shock antigen, on the one hand, and the number and size of the granulomata or of their evolutive and involutive changes and their tendency to conglomerate, on the other. Early granulomata might be found in the guinea-pigs which had received undiluted shock antigen, whereas intratubercular fibrosis with or without degeneration might be seen in the guinea-pigs which had received the more diluted doses. In some fields, the histologic pictures were similar, regardless of the dilution used.

This group of experiments was designed to show graded stages of development of the granuloma. From a histogenetic approach, how-

ever, the experiment is not more adequate than the above experiments or those still to be described.

Group IV

(A) *Specific Antigen, Repeatedly Administered, at Time Intervals of Latency Period Length, Induces Granuloma Formation.* Granulomata, either solitary or conglomerate, were found in varying stages of involution. In some granulomata, intertubercular or intratubercular connective tissue fibrils as well as mucoid degeneration of some of the connective tissue fibers might be seen. Many granulomata displayed giant cells. Lymphocytes and plasma cells were demonstrable, especially at the junction of two or more granulomata, when the latter were incompletely coalesced. There was also plasma cell and monocytic cuffing of capillaries and precapillary arterioles, as well as perineural monocytosis. A recently superimposed mild acute inflammation with the usual marked edema and eosinophilia completed the histologic picture.

(B) *Nonspecific Antigen Repeatedly Administered, at Time Intervals of Latency Period Length, Re-sensitizes the Animal, Becomes Specific, and Induces Granuloma Formation.* Conglomerate granulomata were present, mostly in the stage of mucoid degeneration. There were also perivascular and perineural monocytosis and histiocytosis, without definite clear-cut granuloma-like margins. The remainder of the changes were consistent with a residual inflammation with slight, recently superimposed, acute inflammation and marked eosinophilia. Thus, the changes were identical with those seen in animals A.

Animals A received four subcutaneous injections with specific antigen, counting the original sensitizing dose of egg white. Animals B received only three injections with horse serum. The first injection of horse serum, given 10 days after the initial dose of egg albumin, became a sensitizing injection, while the doses of horse serum given on the 20th and 29th days became shock antigen. Animals B had therefore only two doses of shock antigen (whereas animals A had three) and the action of this antigen upon the focal area was 10 days shorter; yet the changes were similar in both groups.

The experiment proves that "re-sensitization" to a new specific antigen is manifested in its focal response by granulomata, and that while it is possible to annihilate a first sensitization, it still influences the histologic picture by an "additive factor," as shown by almost identical findings in both experiments.

(C) *Various Nonspecific Antigens, Administered at Time Intervals of Latency Period Length, but Never Repeated, Do Not Induce Granu-*

loma Formation. There was no evidence of granulomata, of focal accumulations of histiocytes and monocytes, or of endothelial or perithelial reactivity. Residual inflammation with superimposition of slight and recent acute inflammatory stages was demonstrable. There were occasional perivascular eosinophilic infiltrates.

(*D*) *Nonspecific Antigens Intercalated between Sensitizing and Shock Antigens Do Not Prevent Granuloma Formation.* There were granulomata of varying size. In most of them the histiocytes were transforming into fibroblasts. Alterative changes were conspicuous and exhibited diverse modalities ranging from hyalinization to mucoid and myxomatoid degeneration. The granulation tissue, either in the areas where it harbored granulomata, or in the areas where granu- lomata were absent, showed fair numbers of monocytes and histiocytes. The microscopic picture appeared "too advanced" or "too speeded-up" for an interval of time of only 2 days between the injection of a single dose of shock antigen and the killing of the animals, as well as for animals which had received in all only one shock injection. This may be attributable to a sort of "additive effect" of the previously intercalated nonspecific antigens.

III. The Anaphylactic Granuloma: Morphology, Histogenesis, Evolution, and Involution

The qualitative changes in a focal anaphylactic inflammatory re- sponse manifest themselves in two forms:

1. Histiocytic and monocytic accumulations, which may be irregu- larly shaped, ill-delimited, or streamer-like condensations in the granu- lation tissue. They are unrelated to vessels, nerves, or fat tissue. Such cell aggregates are rather rare and usually found outside the area housing the granulomata. (Diffuse histiocytosis.)

2. Round or ovoid cell accumulations, which vary in size and age, and may be solitary, or fused, encased into similar formations, or conglomerated. They are often well-delimited, and rarely faintly con- toured. They may be encapsulated or not. They are related to nerves, adipose lobules, and mainly to capillaries as well as, in rarer instances, to precapillary arterioles. (Granulomata.)

(a) The perineural granulomata (Fig. 3) are generally nonencap- sulated and have peripherally fading histiocytes and monocytic stream- ers. In cross section they appear rounded. When the nerve is cut obliquely or lengthwise, their shape is irregular, vaguely triangular, with slightly curved, internally concave margins and smoothed angles. In contradistinction to the perivascular granulomata, they are more

readily evanescent and their histiocytes are more prone to undergo fibroblastic metamorphosis. Their histiocytes and monocytes usually do not pierce the perineurium, whereas the polymorphonuclear and, especially, eosinophilic leukocytes almost always invade the nerve.

(b) Granulomata developing in and around adipose lobules (Fig. 2) constitute a variant of the perivascular granulomata, structurally modified by environmental factors. They originate at the expense of the intralobular capillaries, by multiplication and unleashing of the prospective potencies of their endothelial and perithelial cells. The newly engendered cells impinge upon the neighboring fat cells and stem, by diffuse streamers of monocytes and related cells, into the argentaffine networks which house the adipose cells. Later the reactive cells penetrate into the fat-emptied adipose cells, and the lobule is ultimately entirely converted into a granuloma. Some of the peripheral cells may spread outside the lobule into the surrounding granulation tissue.

(c) The perivascular granulomata (Figs. 1 and 4) are by far the most numerous and the most conspicuous reactive cell aggregates. They may occasionally surround a precapillary arteriole (Fig. 2). As a rule, however, the perivascular granulomata are centered, at one or another of their evolutive stages, by a functional or vestigial capillary. This capillary may be stromal, but in most instances is a newly formed capillary of the granulation tissue. In some instances, a nerve and its flanking vessels may be embedded in a mass of granulomatous cells.

Shape. A pericapillary granuloma, when solitary, is more or less regularly circular or ovoid in its outline. Reactive cells from the surrounding granulation tissue may congregate towards a nonencapsulated or already encapsulating granuloma. If they surround concentrically the former layers moulded upon the central capillary, the granuloma will be spheroid. If the reactive cells become apposed to one or both of the spheroid's poles, the shape will assume that of a spheroid surmounted by two cones and later on that of an ovoid. When cells from the granulation tissue are apposed to an encapsulating granuloma, the inner rounded nodule is incarcerated within a larger one; such encased granulomata are not uncommon. Two or more granulomata may come into contact (Fig. 6), or they may fuse together without total loss of their individuality (Figs. 7, 8, and 9). They also may form a larger or coalesced unit, the polycyclic contours of which indicate their multicentric origin. Usually granulomata show a central vestigial capillary, except when the cut surface is a secant to the spheroid granuloma, or when the central capillary has undergone complete necrosis.

Size. Most of the solitary spheroid granulomata measure about

200 μ in diameter. Ovoid single granulomata measure up to about 450 by 250 μ. The largest single granuloma found so far measured 1.5 by 0.5 mm.

Cell Constituents. Histiocytes and Derivatives. Most of the cells are histiocytes, both in actual appearance and in origin. They may show slight variations in type. Some of the cells may lack one or several of the characteristics of histiocytes or monocytes, but they come nearest to such forms. Primitive mesenchymal cells may also be found, scattered among the histiocytes of the nodule. Entirely or predominantly monocytic granulomata are frequent. In occasional granulomata, reticular cells are demonstrable. They rest upon argentaffine fibrils and harbor in their meshes monocytes, giant cells, or syncytia. The enmeshed monocytes may congregate in sheet-like fused elements.

Syncytia, Epithelioid and Giant Cells. Reactive cells, regardless of type, may form small syncytia (Fig. 8) with crowded or piled up nuclei, or larger syncytia when the nuclei are less crowded or separated. The cells may also gather into an epithelioid pattern (Fig. 8) or form giant cells (Fig. 5). Giant cells are usually found at the periphery of the granulomata.

Alterative Changes; Metamorphoses. The cell types may change with the evolutive and involutive phases of the granuloma. It is not uncommon to see them in rows in which the histiocytes which undergo alterative changes are swollen by vacuoles of varying size which give them a foamy character. Before the granulomata undergo fibrosis or degeneration, some of the histiocytes and related cells show fibroblastic metamorphosis. The peripheral histiocytes are transformed into fibroblasts earlier than the central or midzonal elements. Sometimes the marginal fibroblasts, which either delimit or start to encapsulate a granuloma, are extratubercular in origin and stem from the interstitial granulation tissue.

Lymphocytes, Plasma Cells, Eosinophils. In some instances the histiocytes of a granuloma are intermingled with other cells, such as lymphocytes, and, more often, plasma cells. The latter are peripherally located, appear in groups, parallel rows, or, more frequently, in the granulation tissue which separates solitary granulomata (Fig. 13) or incompletely fused granulomata. Lymphocytes and plasma cells usually undergo degenerative changes earlier than the histiocytes. In other instances, when several coalescing granulomata show an almost total mucoid or myxomatous degeneration, the only viable or recognizable cells present are the peripheral or intertubercular plasmacytes. In very rare instances, small granulomata may be entirely plasmacytic.

Eosinophils wander among the granuloma cells. Granulomata with no admixture of eosinophils may also be found.

Vascularization. As a rule granulomata are nonvascular, except for the central capillary, the circulatory function of which is lost very early during the histogenesis of the granuloma. ·In some instances a newly formed capillary courses along an arc of the tubercular periphery and indicates the limit of the granuloma. In other cases a new capillary will branch into intratubercular sprouts which never fan out beyond the outer third of the tubercular area. Penetration of newly formed capillaries into a granuloma usually ushers in fibrosis, although sclerosis may occur also in absence of an intruding vessel, by direct fibroblastic metamorphosis of the histiocytes.

Histogenesis. The initial changes are either predominantly endothelial (Fig. 11) or more markedly (Fig. 10) or even exclusively perithelial (Fig. 1). Simultaneous endothelial and perithelial responses (Fig. 12) are not uncommon and proceed with more or less identical pace in both cell types.

The endothelial cells are swollen and increase up to three to four times their usual size (Fig. 10, and more characteristic in Fig. 12). The nucleus becomes more vesicular, with peripheral, dark, rod-like chromatin strands and a rather clear center; the cytoplasm is slightly darker and in cases basophilic; the cell outlines are precise. Soon the flattened endothelial shape is lost; the cell becomes vaguely fusiform, then more rounded (Fig. 11), and finally sessile or pedunculated. It may rest anchored to the reticular wall or become detached and fall into the lumen. Mitotic figures are frequent in the metamorphosing stage of the endothelial cells as well as in their derivatives. In certain cases most of the endothelial cells respond simultaneously, although the pace of their metamorphosis is not necessarily synchronous. In other cases only one endothelial cell will display reactivity, and the remainder will be quiescent. In a short time the lumen is crowded (Fig. 11) and, in cases, plugged with cells which resemble monocytes, although their cytoplasm may be larger than that of the typical monocyte and the nucleus less indented. Some of the monocytoid forms have cytoplasmic processes. The derivatives of the endothelial cells may be flanked by intraluminal eosinophils. They may phagocytize erythrocytic débris, although this is only rarely seen. Red cell or hemoglobin engulfment, when it occurs, is imperfect or clumsy. No fibrinous thrombi are seen at this or any other stage in capillaries, but may be seen in postcapillary venules. The clump of intraluminal cells does not elicit at any stage a parietal fibroblastic reaction, nor does it

undergo any abortive, involutive, or degenerative process prior to granuloma formation.

The perithelial cells likewise show mitotic figures (Fig. 10) and more frequently than the endothelial cells. Clumps of cells appear at points where usually only one pericyte is expected. The newly formed cells are piled up, sometimes in an epithelial-sheet fashion. The forms are not clear-cut at the beginning; and their evolution, step by step, is more difficult to follow than in the case of the endothelial derivatives. Although they may not be typical histiocytes, they resemble most closely this type, as they display round to flattened cytoplasmic bodies with vaguely ragged outlines and bean-shaped nuclei with inconspicuous nucleoli. Likewise some cell derivatives come nearest to monocytic or original mesenchymal forms, but cannot in all instances be indisputably termed as such. Histiocytic and monocytic patterns predominate, however.

Concomitantly with the endothelial and perithelial metamorphoses, the capillary wall undergoes changes of its own. The various stages range from irregular, blurred staining, fragmented straight or wavy fibril-like structures to a dot-like disintegration. In some cases, when the capillary walls have disappeared, the cells of endothelial ancestry can still be differentiated from those of perithelial origin, the former, mostly monocytic in type, being encased by the latter, which are mainly histiocytic. In subsequent stages, in most instances, this differentiation is no longer possible.

When cell aggregates surround fibrillar remnants of a capillary wall the impression may be gained that the anaphylactic granuloma may have an origin similar to that of an Aschoff body. This impression is false, however. Indeed, (1) all stages ranging from centering capillaries that are intact to capillary wall débris are demonstrable; (2) the dot-like disintegrated walls of the central capillary are reticular and not collagenous; (3) these remnants do not swell, but disintegrate; (4) they do not undergo a fibrinoid degeneration; (5) the reactive cells of a granuloma, even at their earliest evolutive stages, do not show bar-like central nucleoli, conspicuous nuclear vesiculation, or markedly ragged cell outlines; (6) the granuloma at any stage shows more compactness of texture and cell richness than an Aschoff body; (7) the intracapillary and pericapillary origin of a granuloma is in most cases clearly evident in younger granulomata, and traceable in retrospect in older ones.

At this stage of its formation the granuloma is made up of crowded cells, arranged either concentrically or in no definite patterns. Although no marginal fibroblasts are as yet present, the granuloma has a

clear-cut outline, except when it sends out cell streamers. But even in such cases, the bulk of the cell aggregate constitutes an anatomic unit.

At various stages of its development, the granuloma acts as a center of attraction for neighboring cells when these are endowed with identical morphologic and functional characters. Histiocytes and monocytes from the surrounding granulation tissue appear to have pivoted and turned one of their poles towards the periphery of the nodule, to have come nearer to it, and finally to have reinforced the cells of the granuloma.

Encapsulation, Fibrosis, Involution. Involutive changes may be seen at any time, even when the granuloma shows signs of growth. One or several histiocytes, whether peripheral or midzonal, may undergo a fibroblastic metamorphosis. This is heralded by a change in their axis of orientation, which from radial becomes tangential, or by their coursing in a curvilinear direction, with their concavity facing the center of the granuloma. They may lie in single files or in concentric layers, be localized on only one of the sectors of the circumference, or be scattered all around the periphery of the granuloma (Fig. 6). They may or they may not be accompanied by connective tissue fibrils. Encapsulation may therefore be only polar or on an arc of the circumference before becoming circular. In many cases, in which the wrapping collagenous fibers are missing, the encapsulation is only apparent and rather in the nature of a fibroblastic delimitation. Fibroblastic metamorphosis of midzonal histiocytes brings about a delineation of a small circular arc which finally becomes a spheroid encased into the granuloma. Sometimes encapsulation is begun, but new histiocytes which come from the granulation tissue are apposed to the fibroblastic barrier. In the course of time, the granuloma will be either partially or entirely fibrosed.

Fibrosis may or may not be followed by degeneration. This degeneration may be mucoid (Fig. 8) or myxomatoid (Fig. 9). In the mucoid type the fibrils, and later on the cell processes and the fibroblastic bodies as well, take the basic components of the Mallory stain and assume afterwards a greenish hue (pale orange with phosphotungstic acid hematoxylin). Subsequently parts of the granuloma are replaced by greenish patches where vestigial cells are seen, either shriveled or swollen. In the myxomatoid type the cells become separated but are still in contact with their polar or stellate processes; the intervening matrix is ridged, or coarsely granular, or homogeneously swollen and of the same greenish hue (when stained with Mallory's phloxine and methylene blue). The picture suggests Wharton's jelly.

Degenerating granulomata may show a diversified structure. In the

mottled greenish mass of mucoid matrix, the cells may or may not be identifiable. In other cases the degeneration is preceded by the appearance of giant cells or syncytia. Fibrosed or degenerated, the granulomata are always recognizable by their shape, by the concentric or vaguely whorled arrangement of their cell remnants, and by the richness of the circumscribing granulation tissue in histiocytes and plasma cells.

No experiments have been performed to ascertain the length of time necessary for a granuloma to disappear.

SUMMARY

Subcutaneous injections of shock antigen into specifically sensitized guinea-pigs induce a local inflammatory reaction which is at variance with nonanaphylactic ("normergic") inflammation, both quantitatively and qualitatively.

1. The quantitative changes are known from the work of Arthus and Breton, Rössle, Gerlach, and others, and consist of a stormy and intense unfolding of the process with initial ischemia, overwhelming edema, marked polynucleosis, eosinophilia, and early fibrosis as main characteristics.

2. The qualitative changes are characterized by:

A. Complete local resorption of the specific antigen. Whether this resorption is in the nature of a local fixation by either edema or reactive histiocytes is, at this stage of the investigation, only conjectural.

B. A histiocytosis, which may manifest itself:

    I. Rarely in diffuse aggregates of histiocytes and related cells.

    II. Most frequently by granulomata, which are:

        (a) Perineural, the least reactive cell aggregate;

        (b) Perivascular, and especially pericapillary, the best represented and the most reactive cell aggregate;

        (c) Intra-adipose and peri-adipose, an environmentally conditioned variant of the pericapillary granuloma.

3. The morphology, cell constituents, histogenesis, evolution, and involution of the anaphylactic granuloma have been described.

4. The origin of the granulomata is twofold:

A. Endothelial and/or perithelial, through prospective potencies of these cells.

B. In a minor degree, through apposition of histiocytes from the surrounding reactive granulation tissue.

5. The qualitative reaction occurs constantly. Dilution of the shock antigen is immaterial. There are, however, variations with regard to

the intensity of the reaction (number, size of granulomata, their tendency to conglomerate) and to its modalities (presence or absence of plasma cells; epithelioid patterns; giant cells). This variability in intensity and modality of the focal anaphylactic response is at present not attributable to any factor. Such variations may be seen from experiment to experiment, or in cases, from animal to animal within the same experimental group, and even from tissue block to block.

6. The part played by intermingled cells (lymphocytes, plasma cells) is not clear. However, plasma cells are more frequently seen: (a) at the periphery of the granulomata, especially when the nodules fuse or congregate; (b) at the periphery of fibrosing and, especially, degenerating granulomata; (c) in granulomata induced by shock antigen repeated several times at long intervals; (d) in pericapillary mantlings; (e) occasionally as small plasmacytic granulomata.

7. Local injections of nonspecific antigen do not induce granulomata, except when the nonspecific antigen, by repeated injections, at time intervals of latency period length, re-sensitizes the animals and becomes specific (or shock) antigen.

8. Nonspecific antigens, when preceding specific antigens, or when intercalated between the sensitizing and shock antigens, may speed up the histogenesis, evolution, and involution of granulomata, and act as "additive factors" which elicit "additive effects."

9. The anaphylactic granuloma is not patterned histogenetically and histologically after the Aschoff body of rheumatic fever.

REFERENCES

1. Arthus, M. Injections répétées de sérum de cheval chez le lapin. *Compt. rend. Soc. de biol.*, 1903, 55, 817–820.

2. Arthus, M., and Breton, M. Lésions cutanées produites par les injections de sérum de cheval chez le lapin anaphylactisé par et pour ce sérum. *Compt. rend. Soc. de biol.*, 1903, 55, 1478–1480.

3. Fröhlich, A. Ueber lokale gewebliche Anaphylaxie. *Ztschr. f. Immunitätsforsch. u. exper. Therap.*, 1913–14, 20, 476–500.

4. Gerlach, W. Über Beziehungen der Entzündung zum anaphylaktischen Zustand. *Verhandl. d. deutsch. path. Gesellsch.*, 1923, 19, 126–131.

5. Gerlach, W. Studien über hyperergische Entzündung. *Virchows Arch. f. path. Anat.*, 1923, 247, 294–361.

6. Jungeblut, C. W. Die Bedeutung des retikulo-endothelialen Systems für die Infektion und Immunität. *Ergebn. d. Hyg., Bakt., Immunitätsforsch. u. exper. Therap.*, 1930, 11, 1–67.

7. Klinge, F. Untersuchungen über die Beeinflussbarkeit der lokalen Serumüberempfindlichkeit durch Eingriffe am aktiven Mesenchym. *Krankheitsforschung*, 1927, 5, 308 and 458. (Cited by Jungeblut.[6])

8. Rich, A. R. The rôle of hypersensitivity in periarteritis nodosa, as indicated by seven cases developing during serum sickness and sulfonamide therapy. *Bull. Johns Hopkins Hosp.*, 1942, 71, 123–140.

9. Rich, A. R. Additional evidence of the rôle of hypersensitivity in the etiology of periarteritis nodosa. Another case associated with a sulfonamide reaction. *Bull. Johns Hopkins Hosp.*, 1942, 71, 375–379.

10. Rich, A. R., and Gregory, J. E. The experimental demonstration that periarteritis nodosa is a manifestation of hypersensitivity. *Bull. Johns Hopkins Hosp.*, 1943, 72, 65–88.

11. Rössle, R. Über die Merkmale der Entzündung im allergischen Organismus. *Verhandl. d. deutsch. path. Gesellsch.*, 1914, 17, 281–285.

12. Rössle, R. Referat über die Entzündung. *Verhandl. d. deutsch. path. Gesellsck.*, 1923, 19, 18–68.

DESCRIPTION OF PLATES

PLATE 140

FIG. 1. Group I, A. Three granulomata, with intervening area of edema with monocytic infiltration. The upper left granuloma is centered about an arteriole. The lower, although appearing as an anatomic unit, is multicentric. Of note are the widely dilated capillaries and the absence of endothelial reactivities; this is an example of a granuloma of exclusively perithelial origin. Phloxine and methylene blue stain. × 140.

FIG. 2. Group I, A. Peri-adipose and intra-adipose granuloma, early. The lobule is not yet entirely converted into a granuloma. Next to it (separated by lymphatics) two coalescing peri-arteriolar granulomata can be seen. Phloxine and methylene blue stain. × 140.

FIG. 3. Group I, A. Granuloma embedding a nerve and its vascular satellites. Of note is the appositional growth. Phloxine and methylene blue stain. × 140.

FIG. 4. Group I, A. Well outlined, but nonencapsulated granuloma. Phloxine and methylene blue stain. × 140.

FIG. 5. Group IV, A. Small granulomata converted into groups of giant cells. Phosphotungstic acid hematoxylin stain. × 460.
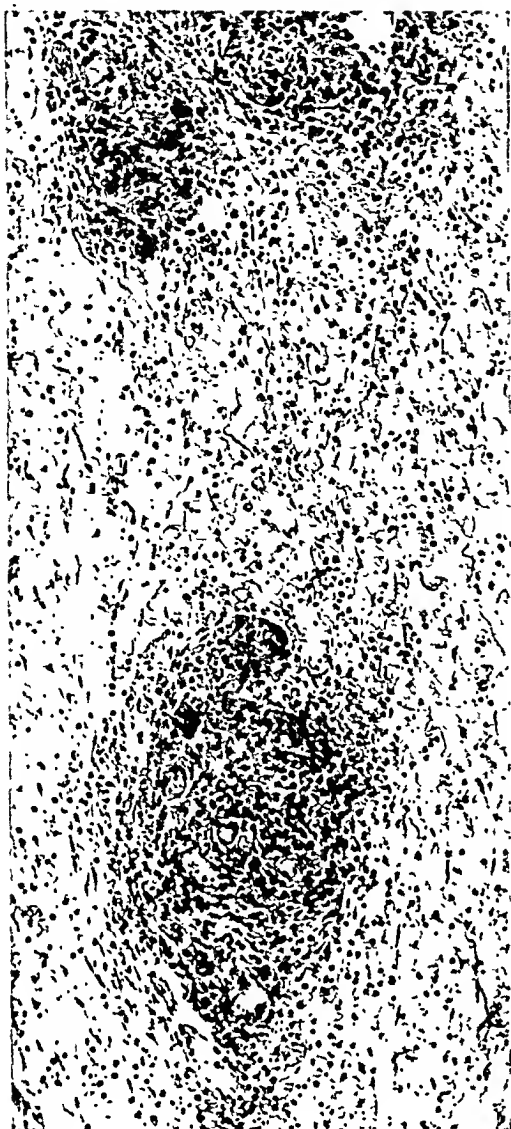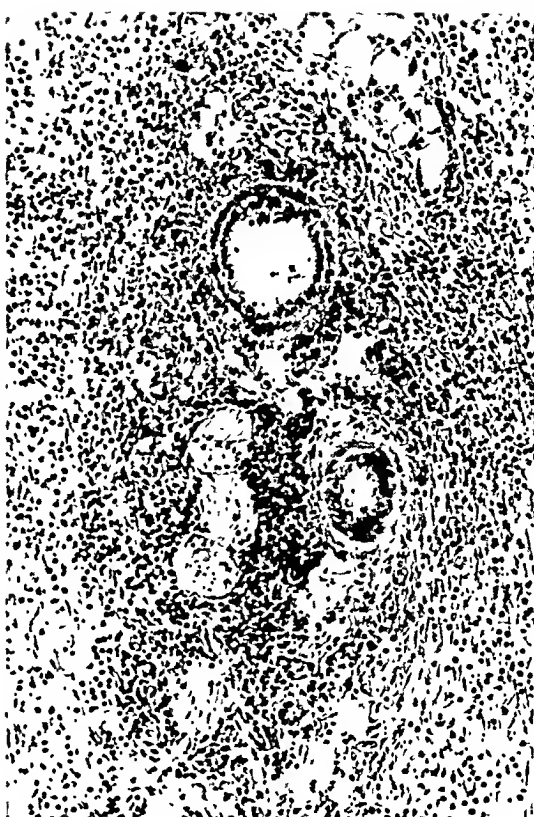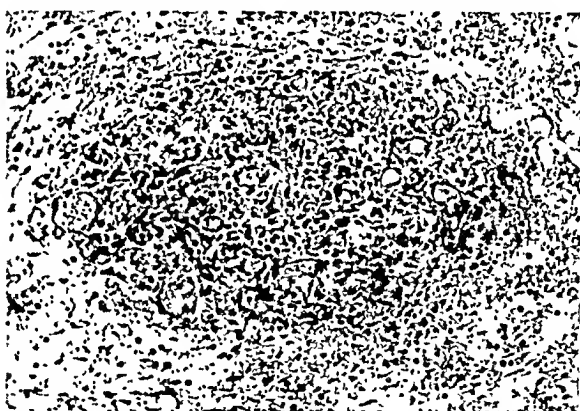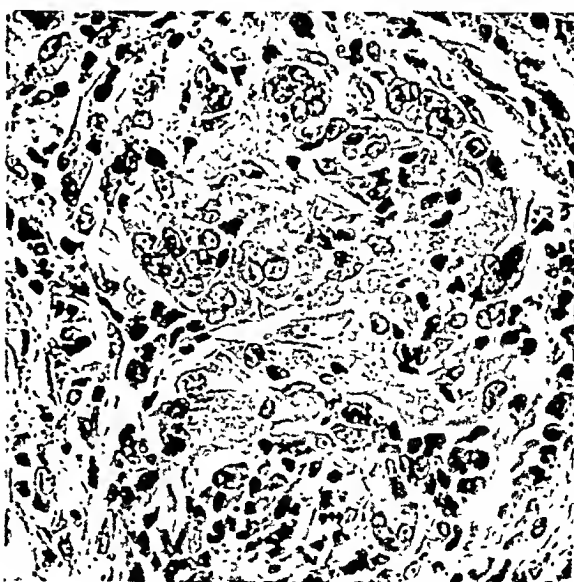
Goddard

Granuloma in Focal Anaphylactic Inflammation

PLATE 141

FIG. 6. Group II, A. Group of granulomata with fibrosing internodular granulation tissue. The upper (and leftwards directed) granuloma shows the vestigial capillary with obliterated lumen (group of whorling cells). In all granulomata there is evidence of actual or impending fibroblastic metamorphosis. Masson's trichrome stain. × 555.

FIG. 7. Group IV, A. Histiocytic granulomata, with compact texture. Phosphotungstic acid hematoxylin stain. × 595.

FIG. 8. Group IV, A. Granulomata and internodular granulation tissue. Upper granuloma made up mostly of histiocytes (fibrillar structure of cytoplasm brought out by Mallory's phosphotungstic acid hematoxylin stain). Vestigial capillary is slightly eccentric. In lower granuloma, where an early, central mucoid degeneration is demonstrable, the marginal histiocytes show an epithelioid pattern and formation of syncytia. Of note is the perinodular fibrosis. Phosphotungstic acid hematoxylin stain. × 595.

FIG. 9. Group II, A. Group of degenerating granulomata. Myxomatoid degeneration (best seen in the granuloma on the left). Phloxine and methylene blue stain. × 280.
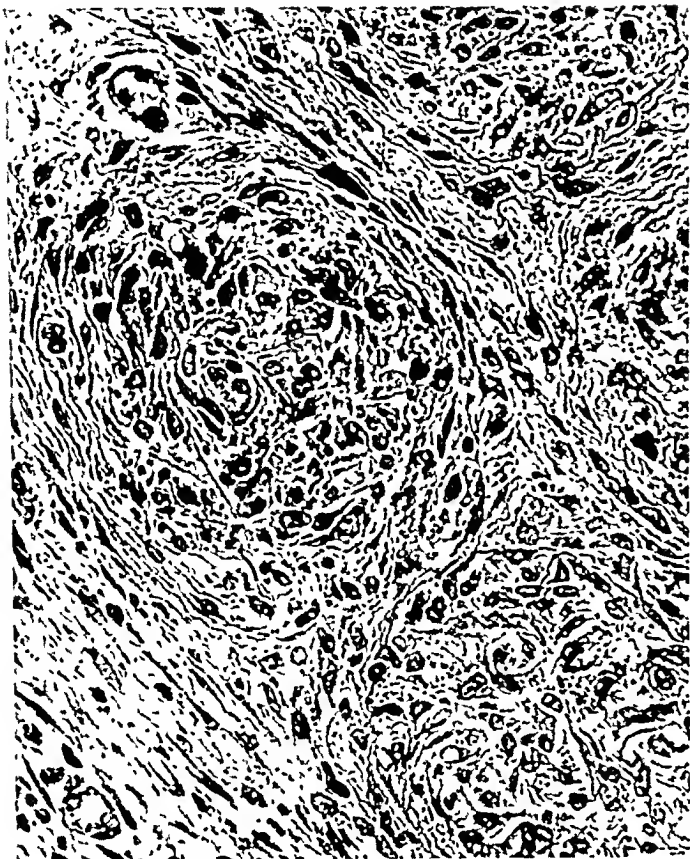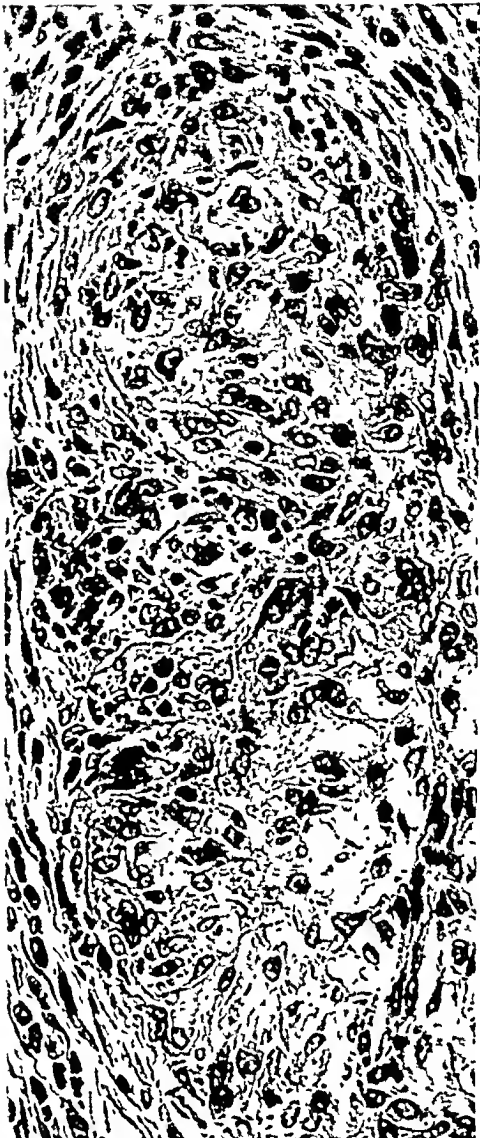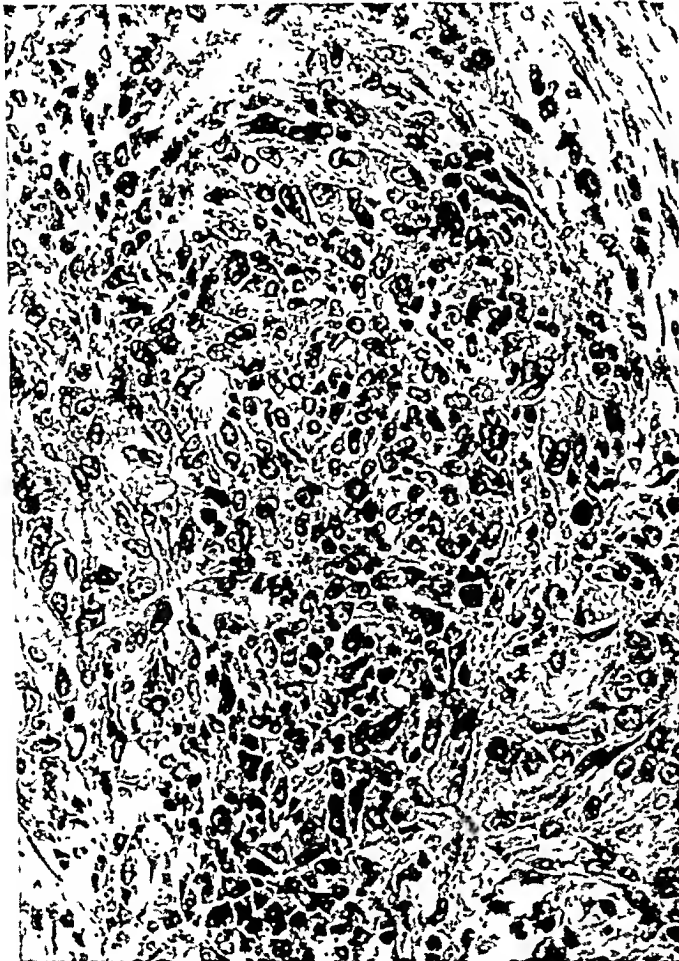
Goddard   Granuloma in Focal Anaphylactic Inflammation

PLATE 142

FIG. 10. Group I, A. Histogenesis of a pericapillary granuloma. Marked perithelial proliferation (several mitotic figures) and metamorphosis (histiocytes, monocytes). Earliest endothelial hyperplasia. Phloxine and methylene blue stain. × 1110.

FIG. 11. Group I, A. Histogenesis. Section through an S-shaped capillary, showing narrowing of the lumen and endothelial hyperplasia, hypertrophy, and metamorphosis. There is also slight perithelial reactivity. Phloxine and methylene blue stain. × 1110.

FIG. 12. Group I, A. Two neighboring capillaries. The capillary towards the left of the figure shows slight to moderate endothelial and perithelial reactivity, but no luminal blockage. The perithelial cells (left margin) are still metamorphosing. The capillary on the right no longer shows its reticulum wall; only a few endothelial cells can be identified; the lumen is crowded with metamorphosing cells. In the lower sector of the figure a vaguely triangular to trapezoidal patch shows the derived cells at almost the end-stage of their metamorphosis. Phloxine and methylene blue stain. × 1110.

FIG. 13. Group IV, A. Plasma cell infiltrate in intertubercular granulation tissue. In places the circular arrangement may give the false impression of plasmacytic granulomata. Phloxine and methylene blue stain. × 742.
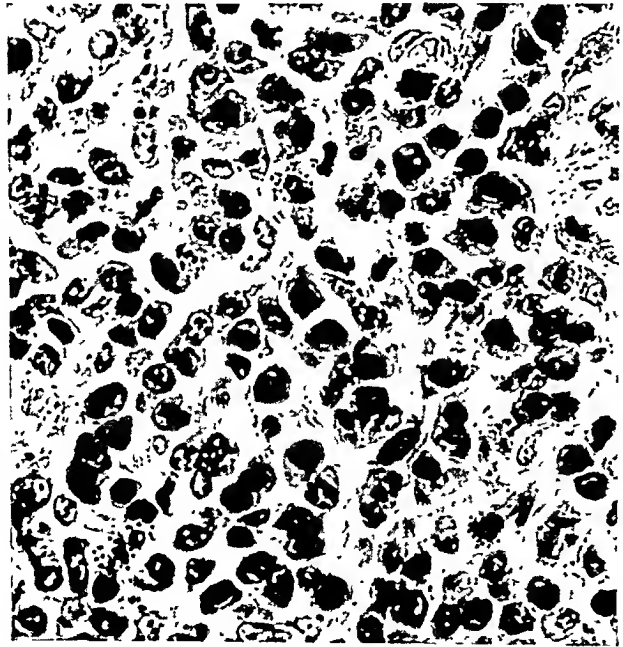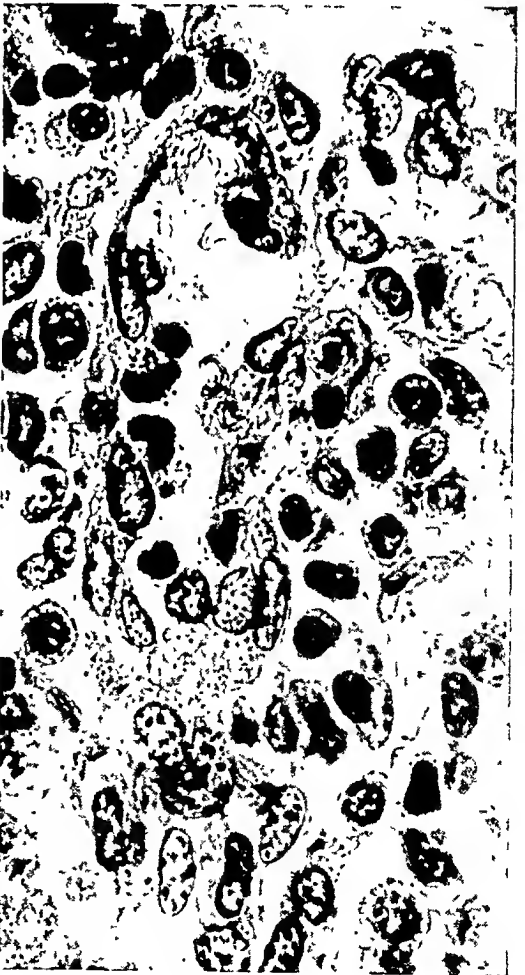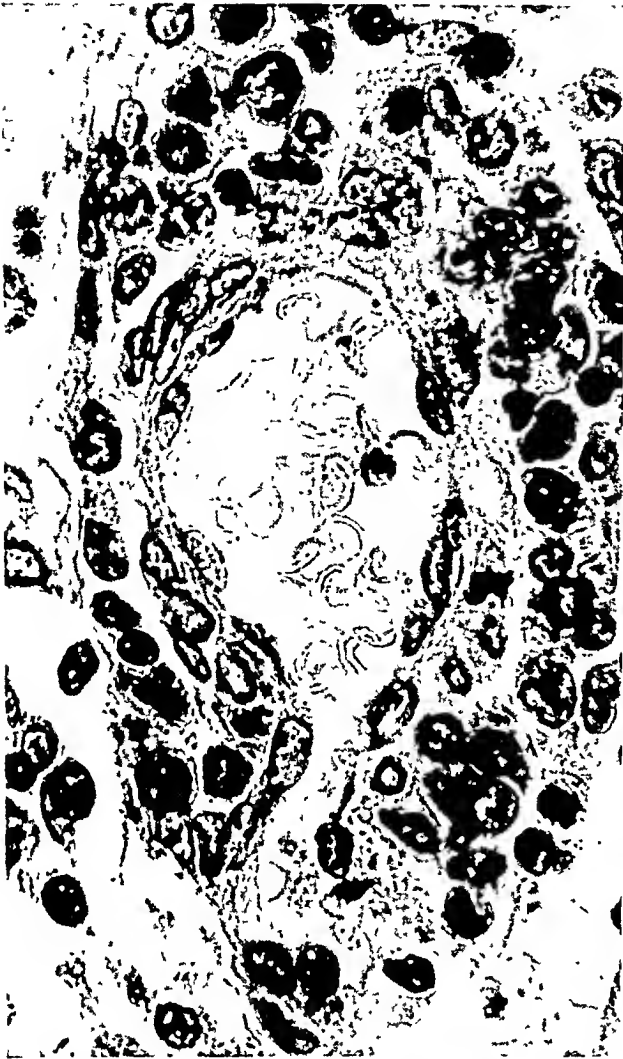
HEMOGLOBIN CRYSTALS, CASTS, AND GLOBULES IN THE RENAL TUBULES OF GUINEA-PIGS FOLLOWING CHEMICAL HEMOLYSIS*

Robert C. Dunn, Surgeon,† and Stewart H. Webster, Senior Biochemist

(*From the Pathology Laboratory and Laboratory of Physical Biology, National Institute of Health, Bethesda, Md.*)

In 1840 Hünefeld [1] observed that bright red plate-like crystals with sharp borders were deposited when the blood of an earthworm was exposed between glass plates. In addition, he referred to crystals from the blood of man and of the pig. Later, in 1849, Reichert [2] reported finding tetrahedral crystals in the fetal membranes and in the mucous membrane of the uterus of a guinea-pig which had died suddenly, and which was examined 6 hours after death. There were four fetuses in the uterus, and in all four placentas the crystals were found. Interest was aroused and reports of other work followed. One of the greatest advances was made by Funke,[3] who in 1851 devised methods of preparing crystals and suggested that the crystals of hemoglobin showed certain physical characteristics which seemed to be related to species. In the next 50 years there were numerous contributions.

In 1911 Miller [4] described crystalline forms of hemoglobin in the capsular space of the glomeruli in a man dying of arsine poisoning. In the same kidney somewhat more delicate crystals were found "in the vicinity of a large artery." He noted in a review of the literature that hemoglobin crystals had been described in the kidneys of dogs and rabbits following poisoning with hemolytic agents or scalding. Since the form of hemoglobin crystals seemed to be different in individual species, including man, he presumed that these differences were explained by the easier crystallization of hemoglobin in many animals, as the horse, dog, and rabbit. This was very soon after the publication of Reichert and Brown [5] in 1909, in which they demonstrated the species specificity of these hemoglobin forms.

Recently one of us (R. C. D.) [6] noted briefly the occurrence of tetrahedral crystals of hemoglobin in the renal tubules of guinea-pigs following chemical hemolysis. Since then these characteristic crystals have been found in many animals of this species, and their staining qualities have been further studied by means of several hemoglobin stains.[6-8]

We have also observed hemoglobin crystals in the renal tubules of rats following massive hemolysis and in rabbits ‡ after experimental

scald burns; and also in the lumen of a hemorrhagic, obstructed uterus * of a rat, and in an intrathoracic lymph node of a mouse. In these species hemoglobin crystals are identified with difficulty because of their complicated structure, but in guinea-pigs they are recognized with ease by their distinct tetrahedral form.

Studies on the mechanism of hemoglobinuria, the renal threshold for hemoglobin, and resorption of this substance by the epithelium of the convoluted tubules have been reported.[9,10] That the amount of fluid in the renal tubules may determine the deposition of hemoglobin has been discussed by Lison.[11]

METHODS AND OBSERVATIONS

In this study, 97 guinea-pigs were subjected to a *single 1-hour exposure* to stibine-air mixtures. Stibine (SbH_3) is a highly toxic gas with hemolytic properties similar to those of arsine (AsH_3).[12] The stibine was prepared by the interaction of a magnesium antimony alloy and dilute hydrochloric acid and the gas mixtures were analyzed for antimony by the rhodamine B method.[13] The guinea-pigs were exposed singly or in groups. While the concentration of stibine was held to fairly close limits during any one exposure, the concentrations employed in the series of exposures ranged from 44 to 293 parts per million (p.p.m.).

Death rarely occurred after exposure at or near the 44 p.p.m. level, but was frequent at 90 p.p.m. or greater. The survival times for animals dying from stibine intoxication varied between 45 minutes and 19 days after exposure. In order to study further the pathologic changes taking place at various time intervals, many of the exposed animals were sacrificed, thus extending the post-exposure time up to 5 weeks and, in a few cases, up to nearly 3 years.

In guinea-pigs succumbing from stibine intoxication during the period of from 2 to 6 days following exposure, the usual clinical findings before death were: dyspnea, lassitude, weakness, anorexia, often hemoglobinuria and a shock syndrome including subnormal temperature and decreased peripheral circulation, and finally coma.

In general, the higher the concentration of stibine, the sooner the beginning and the longer the duration of the hemoglobinuria after exposure. It was observed as early as 45 minutes, usually disappeared within 2 days, and was never found to persist longer than 4 days. During much of the time in which hemoglobinuria was present, hemoglobinemia also occurred, as shown by positive spectroscopic tests for

* Courtesy of Dr. A. A. Nelson, Pathologist, U. S. Food and Drug Administration.

oxyhemoglobin in the plasma. While the presence of hemoglobin in the urine was determined routinely by the benzidine test, this was often checked spectroscopically. Likewise, frequent microscopic tests showed that hemoglobinuria and not hematuria occurred in these animals.

Since hemoglobinuria usually was not present at concentrations below 66 p.p.m. of stibine, 21 guinea-pigs of the experimental animals exposed to less than this concentration are excluded from consideration at this time. Therefore, our main interest centers on the other 76 animals exposed to 66 p.p.m. and above. It should be noted, however, that hemoglobinuria can and has been produced under unusual conditions in a high proportion of guinea-pigs at concentrations less than the above value.

Some of the animals exposed to 66 p.p.m. or more were anuric; in others, experimental conditions did not always permit collection of urine before death. Of the 61 animals from which urine was tested during life, 49 (80 per cent) exhibited hemoglobinuria. On the basis of the benzidine test, the amount of hemoglobin in the urine was marked (4 plus) in about two-thirds of the animals and moderate or slight (3 plus to 1 plus) in the others.

The presence of hemoglobin in the renal tubules is manifested in one of three forms, namely, crystals, casts, or globules, the first two in the lumen and the last in the cytoplasm of the epithelial cells. These hemoglobin deposits were identified by means of their tinctorial reactions in the tissues (Figs. 2 to 6).

The form and tinctorial qualities of these crystals are similar to those of hemoglobin crystals precipitated from the blood of guinea-pigs by a slight modification of the method of Reichert and Brown.[5] Crystallized from the mother liquor by slow evaporation under a cover-slip, the preparations showed tetrahedral crystals, together with truncated and twinned varieties * (Fig. 1).

Hemoglobin crystals were found in the renal tubules of only 23 of 40 guinea-pigs dying or killed during the second to sixth days inclusive, following exposure to 66 p.p.m. or more of stibine (Table I). Of these 40, 23 had both hemoglobin crystals and hemoglobin casts, 3 had casts without crystals, and 14 had neither casts nor crystals, although 2 showed hemoglobin globules. One of the 14 animals, showing only 2 or 3 poorly formed hemoglobin casts, was considered as having none.

Detailed description of the histopathologic findings in the kidneys will be limited to this group of 40 animals, since in the remaining 57

---

* According to Reichert and Brown, crystals of guinea-pig hemoglobin, although appearing to be tetrahedral, have been shown to be orthorhombic sphenoidal in form.

guinea-pigs in this study hemoglobin deposits were seldom found, the incidence for crystals, casts, and globules being 0, 6, and 6 animals, respectively.

PATHOLOGIC FINDINGS IN THE GROUP OF 40 ANIMALS

Upon gross examination the kidneys had the usual appearance associated with intravascular hemolysis. The external as well as the cut surface was dark red, and fine to coarse blackish red striations were often seen in the lighter, outer medullary zone. Occasionally, punctate

TABLE I

Association of Hemoglobin Crystals and Hemoglobin Casts with Hemoglobinuria in 40 Guinea-Pigs Dying or Killed during the 2 to 6 Day Period Following Exposure to 66 Parts per Million or More of Stibine

| Hemoglobinuria | Animals in each group | | | Hemoglobin crystals in the renal tubules | | | | | Hemoglobin casts in the renal tubules | | | | |
| | | | | Number of males | | Number of females | | | Number of males | | Number of females | | |
	Male	Female	Total	Present	Absent	Present	Absent	Total	Present	Absent	Present	Absent	Total
Absent	0	3	3	0	0	0	3	3	0	0	0	3	3
Present	19	8	27	12	7	5	3	27	13	6	6	2	27
Not determined*	9	1	10	6	3	0	1	10	7	2	0	1	10
	28	12	40	18	10	5	7	40	20	8	6	6	40

* Some animals in this classification were anuric; in others, experimental conditions did not permit testing of urine.

blackish red areas were found on the surface under the stripped capsule.

Microscopically, crystals were found in 23 of the 26 guinea-pigs with casts. On the basis of a single 5 μ routine section of tissue fixed in phosphate-buffered neutral formalin and stained by the hematoxylin-picric acid method,[6] each of the 23 animals showed single or grouped crystals in the lumina of 2 to 36 tubules. Using this standardized method, the average number of crystal-containing tubules per animal, arranged according to days (from the 2nd through the 6th), was as follows: 12, 16, 7, 8, and 9.

These crystals had the same tinctorial reactions as the erythrocytes in nearby blood vessels and averaged in over-all length about 20 μ, with extremes of 5 to 54 μ. In formalin-fixed tissue they characteristically had triangular configurations and showed perceptible depth and sharp straight edges (Figs. 2 to 5). However, occasionally it was possible to demonstrate their tetrahedral form. Frequently the edges

showed grooves or lacunae containing a cell or two of mononuclear or polymorphonuclear neutrophilic type (Fig. 6). These cells often formed a part of a conglomerate mass of similar cells that partially or completely enveloped a crystal.

The crystals appeared to be mainly in the convoluted tubules and the loops of Henle in the outer medullary zone. In 2 animals they were noted also in two or three large collecting tubules in the papilla. None was found in the identifiable straight collecting tubules of the medullary rays, and none was found in glomerular spaces.

There was some variation in the number and location of the tubules containing crystals, in relation to the time of death. Arranged in order of successive days following exposure, the ratios of crystal-containing tubules in the cortex (convoluted tubules) to those in the outer medulla (Henle's loop) were as follows: 6:1, 4:1, 4:3, 1:1, and in the single animal dying on the 6th day the medulla was not well seen. The great-- est concentration of crystals occurred in the 3rd day group.

In addition to the crystals, hemoglobin casts (Figs. 3 to 6) were found in 26 of the 40 animals in the 2 to 6 day group. The average number was greatest in the 3rd day group, and they were located chiefly in the convoluted and loop tubules and, rarely, in the large collecting tubules. There was no consistent chronologic relationship between the number of hemoglobin casts found in the tubules of the cortex and the number in the tubules of the medulla.

In the 2nd and 3rd day group of guinea-pigs there were small to large numbers of cytoplasmic hemoglobin globules (Fig. 5) in the convoluted tubular epithelium of 12 of the 18 animals; in the other 22 animals, dead on the 4th to 6th days, only 2 had globules. Small to large amounts of hemosiderin were noted in the cytoplasm of the epithelium lining the convoluted tubules of 37 of the 40 animals, the numbers and the sizes of the granules being slightly greater in the last 3 days.

Dilatation of the tubules was noted in 33 of the 40 animals. In 14, only the tubules of the medullary rays were dilated; in 19, tubules in both cortex and medullary rays were involved. Cytoplasmic basophilia was noted in many of the tubules showing considerable dilatation.

Coagulation necrosis of the epithelium lining convoluted tubules was noted in about half of the 26 animals, the number of affected tubules ranging from about 1 to 5 per section in scattered animals among the series, to a maximum of 20 to 30 in 4 animals in the 3rd and 4th day groups. This necrosis was frequently seen in epithelial cells packed with hemoglobin globules, though not as an invariable accompaniment, and was often found in tubules containing hemoglobin casts or cellular

casts. Occasionally the necrotic cells, along with granular and fibrillar débris, were free in the lumen.

In 23 of 40 animals cellular casts were noted in a few convoluted tubules. In the midst of closely packed mononuclear cells and occasional polymorphonuclear neutrophils, a hemoglobin cast or crystal was often embedded. These cells were often found lying in marginal grooves and, rarely, appeared to be within the substance of the casts or crystals.

In the 26 animals with hemoglobin casts, finely to coarsely globular intracellular fat was noted in large amounts on the 5th and 6th days, but on the 2nd to 4th days there were only traces or small amounts. In the 14 showing no hemoglobin casts there were usually moderate to large amounts of fat on the 3rd through the 6th days, and only traces on the 2nd day. The fat was found mainly in the epithelium of the convoluted tubules and Henle's loops.

Positive evidence of glomerular change was found in but 5 animals. In 2, many of the glomerular tufts were shrunken and bloodless; in 2 others, 6 or 8 glomeruli revealed several capillaries dilated and plugged by dense eosinophilic masses resembling compressed erythrocytes and granular hemoglobin. In the fifth, the glomerular spaces of at least four-fifths of the renal corpuscles were markedly dilated, and in a few instances the first portion of the proximal convoluted tubule was also greatly expanded. It was in this animal that the greatest degree of tubular dilatation was noted.

PATHOLOGIC FINDINGS IN THE KIDNEYS OF THE REMAINING 57 ANIMALS

Examination of the other 57 animals in this study was carried out in somewhat less detail since hemoglobin deposits were so seldom found. Of this number, hemoglobin globules were found in only 2 of the 21 guinea-pigs exposed to concentrations of stibine below 66 p.p.m.; this occurred in those killed on the first day. Hemoglobinuria and casts and crystals were absent in all 21, but hemosiderin was noted in 5. Traces to small amounts of fat were found in 4 of the 10 animals examined.

The remaining 36 animals, not previously discussed, consisted of those exposed to more than 66 p.p.m. of stibine and killed or dying outside the 2 to 6 day period. Of this group, 11 were dead on the first day. Hemoglobinuria was present in 7 of the 8 animals from which urine was obtained. The incidence of hemoglobin crystals, casts, and globules was 0, 3, and 4, respectively. In addition, hemosiderin was found in traces to small amounts in 4 animals, and in moderate amount

in one more. Traces to small amounts of fat occurred in 6 of the 8 animals studied.

The residual 25 guinea-pigs, having survival times of from 7 days to nearly 3 years, showed neither hemoglobin crystals nor globules and only 3 had hemoglobin casts. Fourteen of the 20 animals tested had had hemoglobinuria. Among this group of 25, hemosiderin was found in small amounts in 20, and in moderate amounts in 2 animals. Fat was found in traces to large amounts in 11 of the 17 guinea-pigs examined.

DISCUSSION

Considering the whole group of 97 animals in the study, only after exposure to high concentrations (92 p.p.m. or greater) of stibine was hemosiderin found in moderate amounts in the kidneys of guinea-pigs having survival times of from 3 to 5 weeks. At concentrations lower than this, the hemosiderin was either absent or present in traces.

Animals exposed to concentrations of stibine in excess of 133 p.p.m. usually survived for only a few hours. These generally had hemoglobinuria and sometimes showed hemoglobin casts and globules but no crystals were found under these conditions.

It may be assumed that when hemoglobin is present in the renal tubules, it must become concentrated by some means before crystallization can occur. It is reasonable, likewise, to assume that the structures which we recognize as casts are masses of highly concentrated hemoglobin. The physicochemical state of the hemoglobin in the casts in the 2 to 6 day group of animals evidently fulfills the conditions necessary for crystallization, for there were 23 of the 26 animals with casts (88 per cent) which had crystals.

However, hemoglobin crystals were not always found when hemoglobin casts and hemoglobinuria were present, even in the 2 to 6 day group, for 2 animals had both hemoglobin casts and clinical hemoglobinuria but showed no crystals; and in addition, one animal, in which the ante-mortem urine was not tested for hemoglobin, had casts but no crystals. These 3 animals apparently represent a minority in which the specific physicochemical conditions for crystallization were not present in the tubules. None of the other 14 of the 40 in the 2 to 6 day group had either hemoglobin casts or crystals.

Of the remaining 57 of the 97 animals in this study, 21 were exposed to concentrations of stibine less than 66 p.p.m. Among these hemoglobinuria did not occur and no hemoglobin casts were found, although two showed hemoglobin globules. The residual group of 36 guinea-pigs, dying outside the 2 to 6 day period, had exposures between 66 and 293 p.p.m. of stibine, frequently had hemoglobinuria, and occasionally

exhibited hemoglobin casts. However, in none of these 57 animals were crystals found, regardless of time of survival.

These findings suggest the possible relationships between the concentration of the inhaled hemolytic agent, the survival time, and the deposition of hemoglobin casts and crystals in the kidney. Assuming that hemoglobin crystals cannot form in the renal tubules unless hemoglobin is present in the lumen, there should be close correlation in the incidence of hemoglobinuria, hemoglobin casts, and hemoglobin crystals. These relationships may be observed in Table I. Here in the 2 to 6 day post-exposure group in which hemoglobinuria was present, a total of 27 guinea-pigs, one notes that there were hemoglobin crystals in the renal tubules of 17. In 6 other animals, in which the benzidine test was not done, there were crystals associated with casts. In 3 other guinea-pigs, in which hemoglobinuria was absent, there were neither casts nor crystals. However, one of these showed hemoglobin globules in the renal epithelium.

From a consideration of the data for the 97 animals in this study it is possible to formulate a picture of the events which occur as the animals are exposed to increasing concentrations of the hemolytic gas. The dependence both on concentration and time can be seen from Table II which summarizes the clinical and histopathologic findings with reference to hemoglobinuria and associated phenomena.

The effect of the concentration of the hemolytic gas and the duration of hemoglobinuria is dependent on so many variable factors that it is difficult to predict the amount of hemolysis that is likely to occur in any one animal, or whether hemoglobinuria will be present. There are several generally recognized contributory factors which have a bearing on the deposition of hemoglobin in the kidney during hemolysis, among which are pH of the urine, renal threshold for hemoglobin, and degree of diuresis. The formation of crystals in scattered tubules of the kidney points to a widely variable physicochemical environment in different nephrons during hemoglobinuria. Localized crystal formation in scattered areas in a wet drop, when using the microchemical triple nitrite test for lead,[14,15] presents an interesting analogy.

The relatively great solubility of crystalline guinea-pig hemoglobin may explain why these crystals were never found in the kidneys of these animals beyond the sixth post-exposure day and also why the crystals were so small and frequently appeared to have irregular edges, as if they were in the process of dissolving.

Using stibine as the hemolytic agent, attempts [16] made to demonstrate the presence of crystalline hemoglobin in the kidneys of several other species, such as rats, mice, rabbits, dogs, cats, and monkeys, have

been only occasionally successful. Hemoglobinuria was absent in the rabbit, dog, cat, and monkey; it persisted in the rat for only a few hours; and when present in mice invariably resulted in quick death of the animals. It is believed, therefore, that neither the time nor the concentration was of sufficient magnitude to insure crystallization in the kidneys of mice and rats.

TABLE II

Summary of Clinical and Histopathologic Findings with Reference to Hemoglobinuria and Associated Phenomena

Concentration of hemolytic gas (SbH₃) in parts per million	Presence of hemoglobin in urine	Presence of hemoglobin globules, casts, and crystals in kidneys
44–65	Very infrequent	Globules: infrequent Casts: absent Crystals: absent
66–91	Frequent	Globules: frequent in 1–3 day group; infrequent thereafter Casts: infrequent Crystals: absent
92–133	Almost invariable	Globules: very frequent in 2–3 day group; infrequent thereafter Casts: present in about ¾ of 0–6 day group; about ¼ of 6–9 day group; infrequent thereafter Crystals: present in about 9/10 of 2–6 day group; absent before or after that time

SUMMARY

Single one-hour exposures to stibine, a hemolytic gas similar to arsine, were administered to guinea-pigs, wide ranges of concentration being used with different groups of animals. Hemoglobinuria was observed frequently with concentrations above 65 parts per million.

Under conditions which are described, hemoglobin crystals were formed in the renal tubules of 23 of 97 guinea-pigs. The crystals were found only in animals dying or killed on the second through the sixth day following the exposure to 92 p.p.m. or higher. All of the animals showing crystals had clinical or microscopic evidence of hemolysis, or both. In guinea-pigs succumbing during the above time interval, the usual clinical picture before death was that of dyspnea, weakness, often a shock syndrome, and hemoglobinuria followed by coma.

In addition to crystals, many of the tubules showed hemoglobin casts and globules. The association of these three forms in relation to time and stibine concentration is indicated. Other pathologic conditions, such as tubular dilatation, coagulation necrosis, cellular casts, fat, and glomerular changes, were sometimes noted. Hemosiderosis in

the kidney, although of frequent occurrence, was not found in animals with 3 to 5 weeks of survival time except after exposure to high concentrations of stibine.

There are factors, other than stibine concentration, which may influence the deposition of one or more forms of hemoglobin in the tubules. The data presented are sufficient to give a picture of the chronologic development of hemoglobin globules, casts, and crystals in relation to concentration and time.

REFERENCES

1. Hünefeld, F. L. Der Chemismus in der thierschen Organization. F. A. Brockhaus, Leipzig, 1840, p. 160. (Cited by Reichert and Brown.[5])

2. Reichert, K. E. Beobachtungen über eine eiweissartige Substanz in Krystallform. *Arch. f. Anat., Physiol. u. wissensch. Med.,* 1849, 197–251.

3. Funke, O. Ueber das Milzvenenblut. *Ztschr. f. rat. Med.,* 1851, 1, s. 2, 172–218. (Cited by Reichert and Brown.[5])

4. Miller, J. W. Ueber die Histologie der Niere bei Hämoglobinurie auf Grund elektiver Hämoglobinfärbung. *Zentralbl. f. allg. Path. u. path. Anat.,* 1911, 22, 1025–1034.

5. Reichert, E. T., and Brown, A. P. The Differentiation and Specificity of Corresponding Proteins and Other Vital Substances in Relation to Biological Classification and Organic Evolution: The Crystallography of Hemoglobins. Carnegie Institute of Washington, 1909, no. 116.

6. Dunn, R. C., and Thompson, E. C. A new hemoglobin stain for histologic use. A slightly modified van Gieson stain. *Arch. Path.,* 1945, 39, 49–50.

7. Dunn, R. C. A hemoglobin stain for histologic use based on the cyanolhemoglobin reaction. *Arch. Path.,* 1946, 41, 676–677.

8. Dunn, R. C., and Thompson, E. C. A simplified stain for hemoglobin in tissues or smears using patent blue. *Stain Technol.,* 1946, 21, 65–67.

9. Yuile, C. L. Hemoglobinuria. *Physiol. Rev.,* 1942, 22, 19–31.

10. Monke, J. V., and Yuile, C. L. The renal clearance of hemoglobin in the dog. *J. Exper. Med.,* 1940, 72, 149–165.

11. Lison, L. Zur Frage der Ausscheidung und Speicherung des Hämoglobins in der Amphibienniere. *Beitr. z. path. anat. u. z. allg. Path.,* 1938, 101, 94–108.

12. Webster, S. H. Volatile hydrides of toxicological importance. *J. Indust. Hyg. & Toxicol.,* 1946, 28, 167–182.

13. Webster, S. H., and Fairhall, L. T. The microdetermination of stibine. *J. Indust. Hyg. & Toxicol.,* 1945, 27, 183–192.

14. Behrens, H., and Kley, P. D. C. Mikrochemische Analyse. L. Voss, Leipzig and Hamburg, 1915, ed. 3, p. 28.

15. Fairhall, L. T. Lead studies. VIII. The microchemical detection of lead. *J. Biol. Chem.,* 1923, 57, 455–461.

16. Webster, S. H., Liljegren, E. J., Zimmer, D. J., and Dunn, R. C. Unpublished work.

[*Illustrations follow*]

DESCRIPTION OF PLATES

PLATE 143

FIG. 1. Preparation of guinea-pig blood showing tetrahedral crystals together with truncated and twinned varieties. × 310.

FIG. 2. Small to medium-sized hemoglobin crystals suspended in fibrillar material in the lumen of a dilated tubule. Of note are the two small triangular shaped crystals apparently in the cytoplasm of an epithelial cell lining the tubule at one extremity of the sectioned tubule. × 850.

FIG. 3. Hemoglobin crystals in groups, twinned forms, and singly in one renal tubule. Two hemoglobin casts can be seen, one cut transversely and the other longitudinally. The latter shows thinned, irregular, ragged edges at one end due to beginning resolution. × 390.
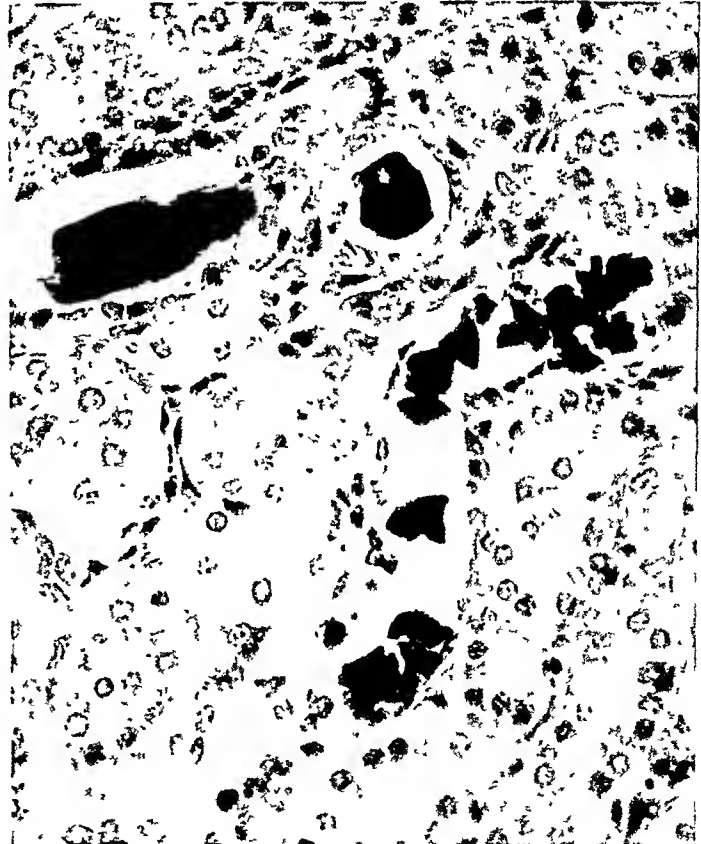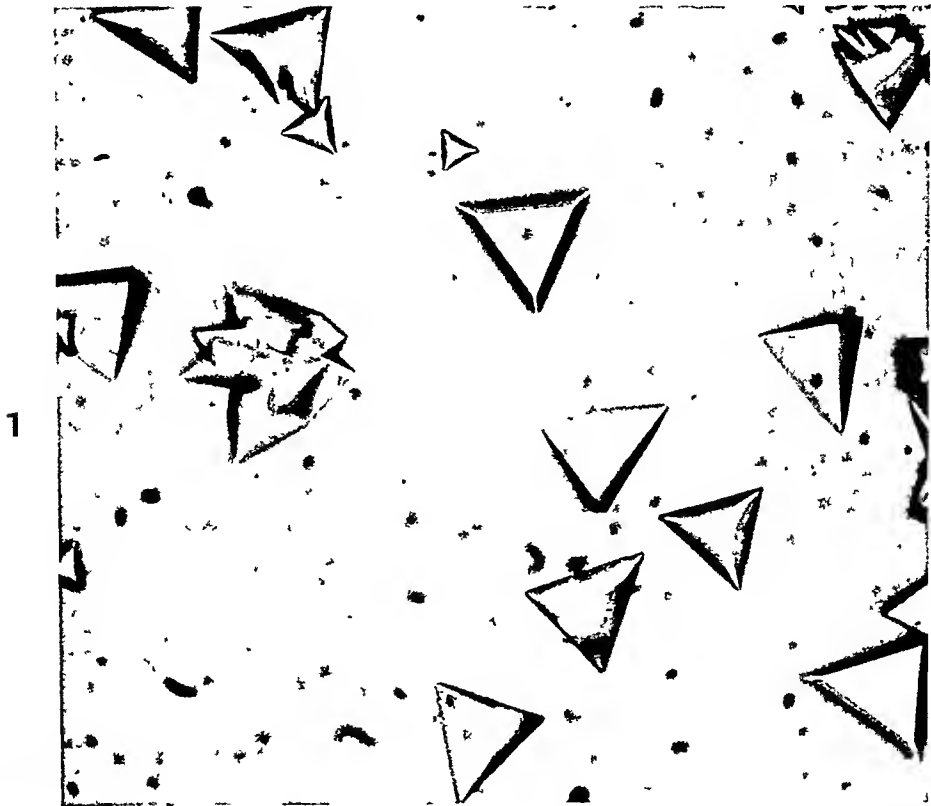
Dunn and Webster

Hemoglobin in Renal Tubules Following Hemolysis

PLATE 144

FIG. 4. Two of the hemoglobin crystals are lying so that the sharp edges are visualized with exceptional clarity. Three large crystalline masses of hemoglobin with angular margins also are seen, one pushed out of the tubular lumen by a wrinkle in the tissue section. $\times$ 350.

FIG. 5. Hemoglobin casts, crystals, and globules in kidney of guinea-pig. The globules are out of focus but visible. One crystal is of truncated configuration. $\times$ 425.

FIG. 6. The process of phagocytosis of both a large cast and the remaining portion of a crystal is fairly well shown. The nuclei of the cells which have invaded the margins can be seen here; microscopically the cells appear to lie within lacunae. One large triangular shaped macrophage contains hemoglobin globules. The glomerulus shows very little change. $\times$ 780.
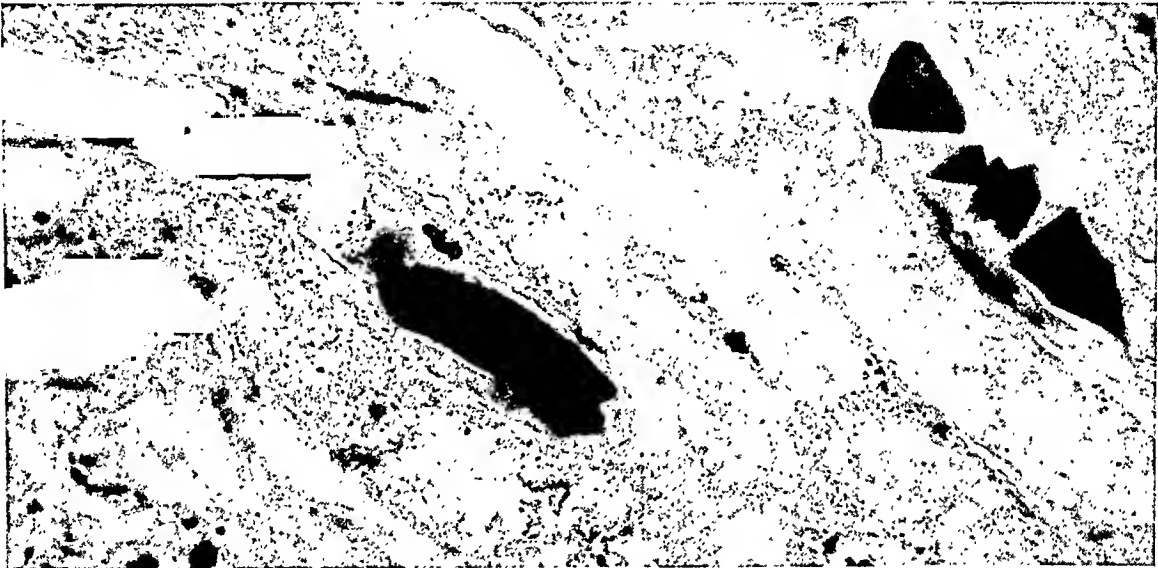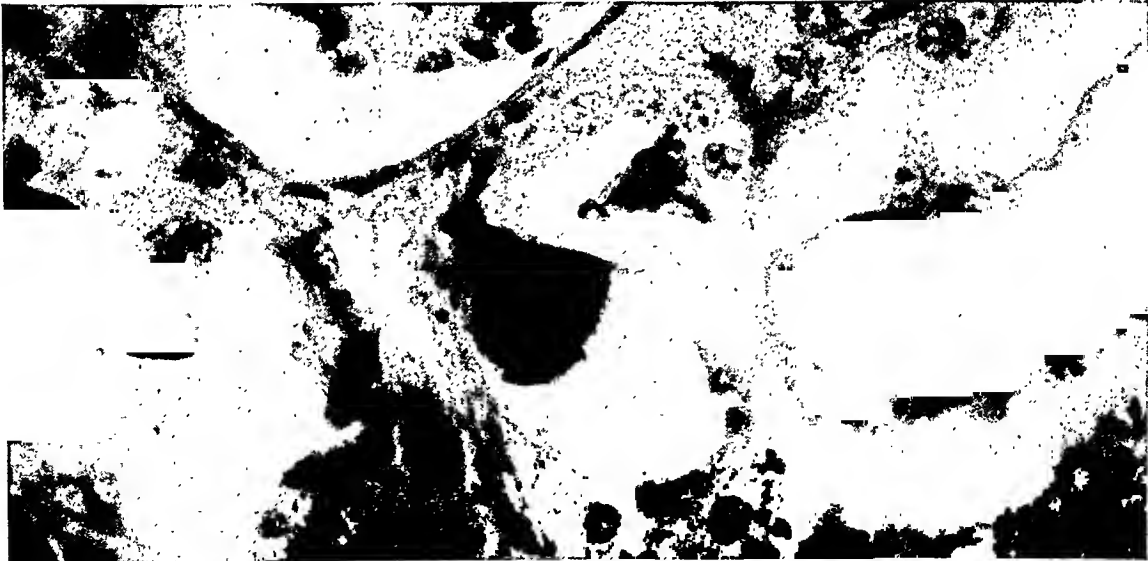
GLOMERULONEPHRITIS OCCURRING IN EXPERIMENTAL BRUCELLOSIS IN DOGS *

George Margolis, M.D., Wiley D. Forbus, M.D., Grace P. Kerby, M.D., and Thomas N. Lide, M.D.

(*From the Department of Pathology, Duke University School of Medicine, Durham, N.C.*)

The production of experimental nephritis has been a problem to which much attention has been given. It has always been difficult to produce, and the results have seldom been consistent with the disease as it is seen in man. Much of the experimental work done in recent years has been directed toward hypersensitivity of renal tissue to some sensitizing agent. Irradiation, high protein diets, and various other forms of injury also have been attempted with varying measures of success.[1-4]

Longcope [5] used horse serum and egg white to produce glomerular lesions in experimental animals, and suggested that nephritis might occur as the result of an allergic reaction.

MacNider [6] thoroughly reviewed the literature up to 1924.

Duval and Hibbard [7] produced acute glomerular lesions in immune and nonimmune rabbits by single large injections of the endotoxic product of the scarlatinal streptococcus, obtained either from the peritoneal lysate of immunized rabbits, or from cultures of the organism treated with activated homologous immune serum. The kidneys in these animals were enlarged, mottled, and showed endothelial proliferation, necrosis, and hyaline thrombi in the glomerular capillaries.

Long and Finner [8] reported the occurrence of proliferative lesions in the endothelial and epithelial elements of glomeruli, with atrophy of the tubules in kidneys of sensitized swine, following the injection of tuberculin into the renal arteries.

Blackman, Brown, and Rake [9] used autolysates of type I pneumococcus to produce lesions in the kidneys of rabbits.

Lukens and Longcope [10] noted the occurrence of hyaline thrombi in the capillary loops with epithelial proliferation in the glomeruli of rabbits in which there was injected into the renal artery a heat-killed vaccine of beta hemolytic streptococcus. Lesions in their animals occurred in 74 per cent of rabbits which had been previously sensitized by intradermal injections of the organism, while lesions occurred in 28 per cent of the nonsensitized rabbits.

Bell and Clawson [11] reported the case of a monkey (*Macacus rhesus*) into which were injected intravenously suspensions of live

streptococci over a period of 4 years. This animal showed in its kidneys involvement of nearly all glomeruli. The glomerular capillaries were narrowed and occluded by endothelial proliferation and by thickening of the basement membrane of the glomerular loops. There was little fat in the tubules, but atrophy was marked.

VonGlahn and Weld [12] found, in the kidneys of rabbits injected with staphylococcus toxin, congestion, distention of the glomerular capillaries, endothelial necrosis with fibrin thrombi, and changes in the proximal convoluted tubules which varied from cloudy swelling to complete necrosis.

Extensive hemorrhagic cortical necrosis was noted by Rigdon, Joyner, and Ricketts [13] in the kidneys of rabbits injected intravenously with *Staphylococcus aureus* toxin. They found in the less involved kidneys of their animals proliferation of the endothelial lining of the glomerular loops and, in some, hyaline thrombi. The involvement of the proximal convoluted tubules and Henle's loops was extensive, ranging from cloudy swelling to complete cellular disintegration.

More recently investigation of experimental renal lesions has emphasized the nephrotoxic or anti-kidney immune aspect. However, Lindemann [14] had in 1900 produced marked albuminuria, cast formation, and tubular degeneration in rabbits by injecting the serum of guinea-pigs which had been immunized to rabbit kidney. Other experiments along the same line followed.[15] In 1936 Smadel [16] reviewed the literature and reported his work in which anti-kidney serum was prepared by immunizing rabbits with perfused rat kidney and injecting the rabbit serum into rats. He found that specific kidney antibodies, as well as antibodies to other tissues, resulted in lesions or organs other than the kidney.

Horn [17] reviewed the literature up to 1937, covering most of the experimental work done since MacNider's review in 1924. Schwentker and Comploier [18] were the first to prepare a nephrotoxic substance from homologous species. They used both *Staph. aureus* and beta hemolytic streptococcus mixed with rabbit kidney and succeeded in demonstrating antibodies to kidney in rabbits into which this kidney-bacterial mixture was injected. They also demonstrated antibodies to kidney in the blood of 37 of 40 scarlet fever patients tested. They offered the interesting explanation concerning the pathogenesis of glomerulonephritis that the combination of the bacteria or their products with kidney tissue results in some alteration of the renal protein, and that this altered protein constitutes a new antigen as it is released into the circulation during the process of repair. The antibody thus produced is capable of reacting with the altered kidney and possibly with nor-

mal kidney. They thus explain the delay in the human species between infection and the onset of nephritis. Kay [19] explained on this basis the delayed appearance of nephritis in rabbits following injection of the serum of ducks immunized to rabbit kidney.

Cavelti and Cavelti [20-22] also produced glomerular and tubular lesions and demonstrated by serologic methods antibodies to kidney in rats and rabbits injected with homologous kidney and bacterial suspensions.

Simonds and Hepler [23] in recent papers have described the kidneys of dogs following injections of heavy metals (potassium dichromate, mercuric chloride, and uranyl nitrate); snake venoms; diphtheria, staphylococcus, and streptococcus toxins.

The production of specific renal lesions by organisms of the brucella group has not been described in reports on canine brucellosis nor in reports on experimental nephritis. Feldman, Bollman, and Olson,[24] in reporting experiments in brucellosis of dogs, noted in many of their animals the clinical symptoms of disease of the kidney, bladder, and prostate, but were inclined to discount its relation to brucellosis, inasmuch as spontaneous infection in the genitourinary tract is frequent in the dog. At autopsy diffuse cortical scarring was found in the kidneys of these animals. Multiple yellowish nodules in the kidney were described by Thomsen,[25] but no microscopic description is included in his monograph. The kidneys were not found to be involved by others who have described the anatomic lesions of brucellosis in dogs.[26-28]

During the course of experiments performed in this laboratory to determine the effect of organisms of the brucella group on the reticuloendothelial system of dogs and to determine the length of time after infection that the organisms could be recovered from the blood stream, viscera, and lymph nodes, there were found in the kidneys of 4 of 9 animals unusual and interesting lesions. The general histopathologic and bacteriologic observations in these experiments have been reported elsewhere.[29,30] The experimental procedures may be reviewed briefly.

MATERIALS AND METHODS

Two strains of *Brucella suis* were selected for inoculation into the test animals. Strain A (ABF 36) was isolated from the spleen of a naturally infected hog. It was virulent for guinea-pigs, producing gross lesions and often death in 3 to 4 weeks after intraperitoneal inoculation. Strain B was obtained at autopsy from a case of Hodgkin's disease, and its virulence for guinea-pigs at the time of these experiments was slight. This has been previously referred to as the Brody strain.[31]

Nine healthy dogs were employed. Seven were used for a study of the infection produced by strain B. In 4 of this group the organisms were inoculated intravenously, while in the other 3 the inoculation was intraperitoneal. In the remaining 2 dogs strain A (ABF 36) was inoculated intravenously in one and intraperitoneally in the other. Each injection consisted of 10 billion organisms.

The inoculations were made at intervals of about 1 week, and in some instances they were given in two series, with time allowed for recovery of the animal in order to prolong the experiment, which varied in the individual animals from 186 to 487 days. One animal included here died from ingesting pine shavings on the 38th day.

The dogs were bled frequently from the jugular vein preceding inoculations. Blood cultures, brucella agglutination titers, and opsonocytophagic indices were determined at each bleeding. The details of the bacteriologic observations were fully described previously.[30]

Of the 4 dogs under consideration here, 2 were severely ill at the time of their deaths, on the 198th and 261st day, respectively, of the experiment. In both animals the Brody organism was inoculated intravenously, and inoculations were continued until death in order to produce an overwhelming disease. The first animal (dog 1) received 21 injections, the last injection 14 days before death. Twelve positive blood cultures were obtained from this animal, the last 7 days before death. In the other animal (dog 4) 28 injections were given, with 23 positive blood cultures. The last injection and the last positive blood culture occurred 7 days before death. At autopsy positive cultures of *Br. suis* were obtained from the spleen, liver, and kidney in each animal and from the lymph nodes of dog 1 and the testis of dog 4.

The other 2 animals (dogs 2 and 3) offered a contrast to the first 2 in the degree of clinical illness. Dog 2 received 35 intravenous inoculations of the Brody organism over a period of 37 weeks, during which time it was severely ill. The injections were discontinued and were followed by clinical recovery until the animal was killed on the 487th day of the experiment. Altogether, 18 positive blood cultures were obtained from this animal, the last 233 days before death. At autopsy *Br. suis* was isolated from the lymph nodes and kidney.

In the next animal (dog 3) 39 intraperitoneal injections were given over a period of 45 weeks. The animal was killed on the 461st day of the experiment, 143 days after the last injection. Only 4 of 41 blood cultures were positive, the last 270 days before death. No positive cultures were obtained from the organs at autopsy. During life this dog showed no signs of peritonitis and was not clinically ill at any time.

RESULTS

The general lesions have been described previously, and only the kidneys will be considered here.

In dog 1 focal epithelioid granulomata attributed to the brucella infection were found in the kidney. There were also widespread glomerular lesions, which consisted of extensive fibrosis and hyalinization of the capillary loops with proliferation of the capillary endothelium, and occlusion of some of the glomerular loops. Necrosis was prominent, with an exudate of polymorphonuclear leukocytes and lymphocytes into the basement membrane of many of the fibrotic glomerular loops. In some instances fibrin, albuminous fluid, and cellular débris were found in the glomerular spaces. Adhesions were numerous, and there was usually a definite thickening of Bowman's capsule. Epithelial proliferation was not prominent, but it did occur minimally in many of the glomeruli. In a moderate degree, dilatation and atrophy were found in the tubules.

The lesions found in the kidneys of dog 4 were similar to those just described. They were, however, more acute, with more prominent necrosis and cellular exudation. Hemorrhage into the glomeruli was occasionally found. Cloudy swelling, dilatation, and atrophy of the tubules were prominent. In addition to the changes described there were several linear collections of mononuclear cells, such as are found in spontaneous nephritis in the dog. These appeared to be unrelated to the glomerular lesions.

In the other 2 animals the glomerular lesions were somewhat different from those just described. In dog 2, capillary dilatation with hyaline capillary thrombi and glomerular hemorrhage were found. Endothelial proliferation and increased cellularity of the glomerulus were prominent. Adhesions between glomerular loops and capsule were present but were not numerous. There were many glomeruli which showed a marked diffuse sclerosis, sometimes with thickening of Bowman's capsule, though more often with no capsular change evident. Epithelial proliferation was not a prominent feature. Cloudy swelling in the tubular epithelium was of moderate degree. Vascular changes were not evident. In dog 3 the changes were similar to those just described. No interstitial inflammation was found in either.

COMMENT

The renal lesions which occurred in these 4 dogs are interesting from the standpoints of experimental nephritis and of chronic brucellosis in the dog. It was demonstrated that *Br. suis* can remain viable over

periods of 5 to 8 months in the tissues of an animal highly refractive to it, and only by repeated massive infection can it produce the disease.

In 2 animals with severe and active disease, active inflammatory lesions were found in the glomeruli. The recent lesions here were super-imposed on chronic glomerular scarring, which was obviously not of recent occurrence. In these animals the nature of the experiment was such that one would expect to find a focal embolic glomerulonephritis, such as occurs in bacteremias in man. The lesions here were similar. They probably were the result of repeated sublethal injury to the glomerulus, as is indicated by variations in age and type of the changes in the capillary loops. Some were sclerotic, while others in the same glomerulus were of more recent occurrence with cellular exudation and necrosis. The changes in the tubules were those usually associated with long-standing glomerular injury.

The lesions in the glomeruli of the other 2 dogs are more difficult to interpret. These dogs had had repeated injections early in the course of the experiment; and, although one (dog 2) had been severely ill, it had recovered clinically and had received no injections of the organisms for 8 months prior to death. The other (dog 3) had received 39 intraperitoneal injections, the last injection 5 months prior to death. This animal had not been clinically ill and no organisms were recovered from its tissues at autopsy. The organisms were recovered from lymph nodes and kidneys of the former. There apparently was a progressive renal lesion, histologically more nearly analogous to that of an acute and subacute glomerulonephritis such as is seen in man, and different in structure from the lesions found in the kidneys of the first 2 dogs. It should be emphasized that these lesions differ from the spontaneous interstitial nephritis so common in the dog.[32]

In dog 2 a persistent brucella infection was demonstrated in the kidneys and lymph nodes, while no such infection could be found in the tissues of dog 3. Five of the 9 dogs showed no glomerular lesions. There was no correlation between the level of the agglutination titers to *Br. suis* or the opsonocytophagic index and the occurrence of renal lesions. All titers were high during the entire experiment. Moreover, no correlation could be found between the nephritic lesions and the positive blood cultures obtained in the last 2 animals. Such was not the case with the severely ill and repeatedly infected dogs showing the embolic glomerular lesions. In the last 2 animals (dogs 2 and 3) the mechanism of renal injury is probably that of renal injury of similar type in man, whether it be direct or due to an altered reactivity of the tissues.

There are certain data which would be of interest but which are not

available here. The serologic investigation of antibodies to kidney in the circulating blood during the experiment, and biopsies of the kidneys from time to time should be a part of any future work along these lines.

SUMMARY

1. In experimental brucellosis of dogs there occurred in 4 of 9 animals renal lesions of a type not usually found in dogs.

2. In 2 of the animals necrotizing acute inflammatory lesions involving portions of the glomerulus were found to be superimposed on an older sclerotic glomerulonephritis, such as is seen in focal acute glomerulonephritis in man.

3. In other dogs the lesion was a progressive, active, subacute glomerulonephritis, somewhat similar in structure to the lesion found in man.

REFERENCES

1. Bolliger, A., and Laidley, J. W. S. Experimental renal disease produced by x-rays: Histological changes in the kidney exposed to a measured amount of unfiltered rays of medium wave length. *M. J. Australia*, 1930, 1, 136–147.

2. Page, I. H. Production of nephritis in dogs by roentgen rays. *Am. J. M. Sc.*, 1936, 191, 251–256.

3. Drummond, J. C., Crowden, G. P., and Hill, E. L. G. Nutrition on high protein dietaries. *J. Physiol.*, 1922, 56, 413–420.

4. Watson, C. Food and Feeding in Health and Disease. A Manual of Practical Dietetics. William Wood & Co., New York, 1913, ed. 2, p. 578.

5. Longcope, W. T. The production of experimental nephritis by repeated proteid intoxication. *J. Exper. Med.*, 1913, 18, 678–703.

6. MacNider, W. deB. A review of acute experimental nephritis. *Physiol. Rev.*, 1924, 4, 595–638.

7. Duval, C. W., and Hibbard, R. J. Experimental production of acute glomerulonephritis. Use of active principle of scarlatinal streptococcus and a consideration of chronic interstitial changes: preliminary report. *J. A. M. A.*, 1926, 87, 898–901.

8. Long, E. R., and Finner, L. L. Experimental glomerulonephritis produced by intrarenal tuberculin reactions. *Am. J. Path.*, 1928, 4, 571–586.

9. Blackman, S. S., Brown, J. H., and Rake, G. The production of acute nephritis by means of a pneumococcal autolysate. *Bull. Johns Hopkins Hosp.*, 1931, 48, 74–89.

10. Lukens, F. D. W., and Longcope, W. T. Experimental acute glomerulonephritis. *J. Exper. Med.*, 1931, 53, 511–526.

11. Bell, E. T., and Clawson, B. J. Experimental glomerulonephritis in a monkey. *Am. J. Path.*, 1931, 7, 57–61.

12. VonGlahn, W. C., and Weld, J. T. The effect of *Staphylococcus aureus* toxin on the kidney. *J. Exper. Med.*, 1935, 61, 1–8.

13. Rigdon, R. H., Joyner, A. L., and Ricketts, E. T. A study of the action of a filtrable staphylococcal toxin on the kidneys of normal rabbits. *Am. J. Path.*, 1934, 10, 425–433.

14. Lindemann, W. Sur le mode d'action de certains poisons rénaux. *Ann. Inst. Pasteur*, 1900, 14, 49–60.

15. Pearce, R. M. An experimental study of nephrotoxins. *Tr. A. Am. Physicians*, 1903, 28, 557–598.

16. Smadel, J. E. Experimental nephritis in rats induced by injection of anti-kidney serum. I. Preparation and immunological studies of nephrotoxin. *J. Exper. Med.*, 1936, 64, 921–942.

17. Horn, H. The experimental nephropathies. *Arch. Path.*, 1937, 23, 71–121; 241–264.

18. Schwentker, F. F., and Comploier, F. C. The production of kidney antibodies by injection of homologous kidney plus bacterial toxins. *J. Exper. Med.*, 1939, 70, 223–230.

19. Kay, C. F. The mechanism of a form of glomerulonephritis; nephrotoxic nephritis in rabbits. *Am. J. M. Sc.*, 1942, 204, 483–490.

20. Cavelti, P. A., and Cavelti, E. S. Studies on the pathogenesis of glomerulonephritis. I. Production of autoantibodies to kidney in experimental animals. *Arch. Path.*, 1945, 39, 148–152.

21. Cavelti, P. A., and Cavelti, E. S. Studies on the pathogenesis of glomerulonephritis. II. Production of glomerulonephritis in rats by means of autoantibodies to kidney. *Arch. Path.*, 1945, 40, 158–162.

22. Cavelti, P. A., and Cavelti, E. S. Studies on the pathogenesis of glomerulonephritis. III. Clinical and pathologic aspects of the experimental glomerulonephritis produced in rats by means of autoantibodies to kidney. *Arch. Path.*, 1945, 40, 163–172.

23. Simonds, J. P., and Hepler, O. E. Experimental nephropathies. I. A method of producing controlled selective injury of renal units by means of chemical agents. *Arch. Path.*, 1945, 39, 103–108.

24. Feldman, W. H., Bollman, J. L., and Olson, C., Jr. Experimental brucellosis in dogs. *J. Infect. Dis.*, 1935, 56, 321–332.

25. Thomsen, A. Brucella infection in swine. Studies from an epizootic in Denmark 1929–1932. *Acta path. et microbiol. Scandinav.*, 1934, suppl. 21, pp. 9–242.

26. Plantz, J. F., and Huddleson, I. F. Brucella infection in a dog. *J. Am. Vet. M. A.*, 1931, 79, 251–252.

27. Davis, C. L. A clinical case of brucellosis in a dog. *North Am. Vet.*, 1937, 18, 48.

28. van der Hoeden, J. Over spontane en experimenteele brucella-infectiebij den hond. *Tijdschr. v. diergeneesk.*, 1932, 59, 1383–1396.

29. Margolis, G., Forbus, W. D., and Kerby, G. P. The reaction of the reticulo-endothelial system in experimental brucellosis of dogs. *Am. J. Path.*, 1945, 21, 753–777.

30. Kerby, G. P., Brown, I. W., Jr., Margolis, G., and Forbus, W. D. Bacteriological observations on experimental brucellosis in dogs and swine. *Am. J. Path.*, 1943, 19, 1009–1020.

31. Forbus, W. D., and Gunter, J. U. The pathogenicity of strains of brucella obtained from cases of Hodgkin's disease. *South. M. J.*, 1941, 34, 376–389.

32. Bloom, F. Classification and pathology of renal disease in the dog. Comparison with nephritis in man. *Arch. Path.*, 1939, 28, 236–245.

[*Illustrations follow*]

DESCRIPTION OF PLATE

PLATE 145

FIG. 1. Dog 1. Extensive acute inflammatory reaction in the lower glomerulus, with scarring and obliteration of the capillary loops, particularly evident in the upper glomerulus. × 265.

FIG. 2. Dog 4. The necrotic lesion with fibrinoid changes, seen in many of the glomeruli of this kidney. × 265.

FIG. 3. Dog 2. Thickening of the capillary basement membrane and endothelial proliferation are characteristic of the changes found in this animal. × 265.

FIG. 4. Dog 3. The changes here are those of endothelial proliferation, scarring, and focal necrosis with glomerular hemorrhage. They are similar to the changes shown in Figure 3. × 325.
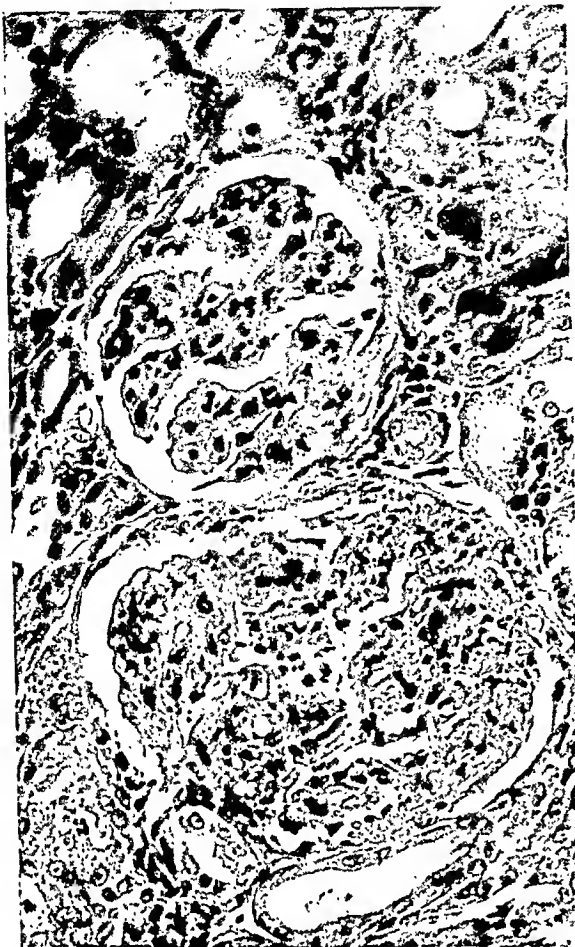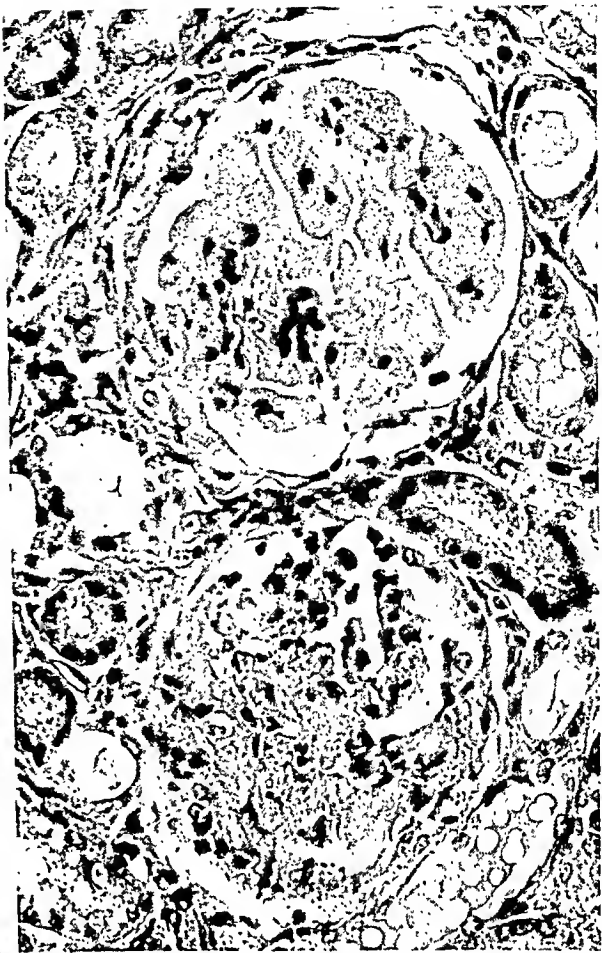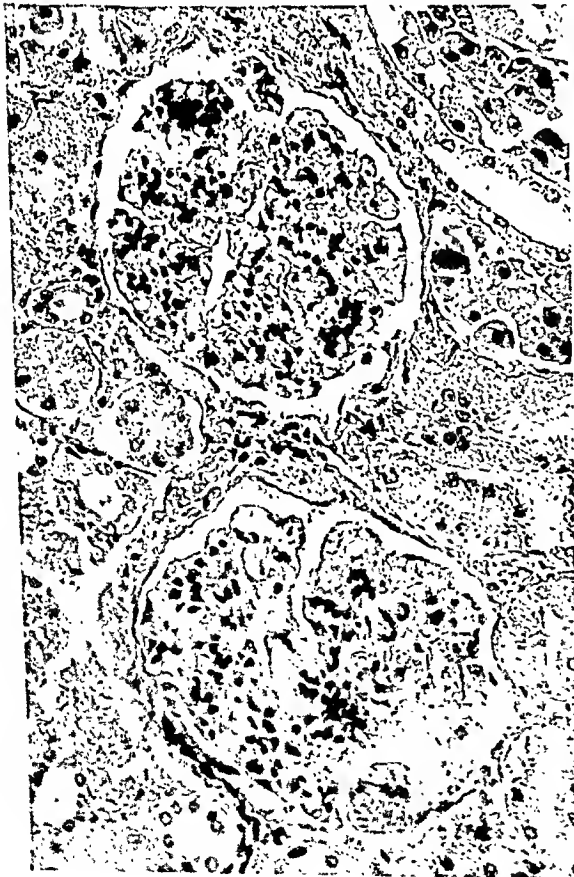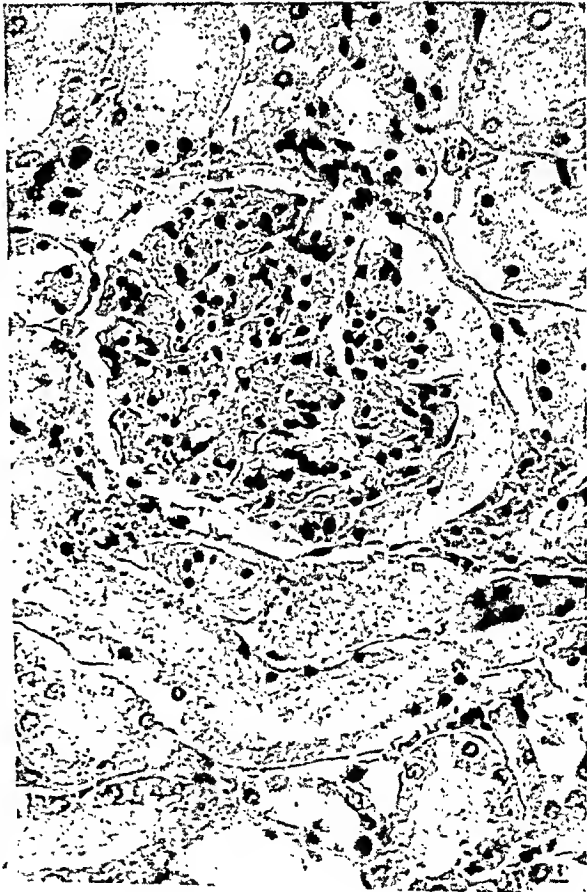
FATAL INCLUSION-DISEASE PNEUMONITIS IN AN ADULT *

GARDNER C. McMILLAN, M.D. †

(*From the Department of Pathology, Pathological Institute, McGill University, Montreal, Que.*)

While occasional cases of inclusion disease affecting various organs in infants have been reported for many years, the condition remains distinctly rare in adults. The following case represents, so far as I am aware, the ninth to be reported in an adult, and the second example of adult inclusion-disease pneumonitis.

REPORT OF CASE

The patient was a Japanese female, 60 years old, who was admitted to the Royal Victoria Hospital, Montreal, on the service of Dr. J. F. MacIntosh, on June 18, 1945. The admission complaints comprised difficulty in breathing for 7 weeks following a cold, and diarrhea for 7 weeks. The patient's personal history was non-contributory; she had been born in Japan, but had lived in Canada for 32 years. The functional inquiry elicited that about 4 weeks before admission her face had become hot and flushed during the afternoon and her wrists and ankles had become erythematous. Weeping eruptions had occurred at the wrists and ankles without pain or pruritis, but these had regressed somewhat at the time of admission.

On physical examination the temperature was 100° F., pulse, 88; respirations, 35; blood pressure, 96/64 mm. Hg. There was a skin eruption over both wrists and ankles, extending up the forearms and the back of the left leg to the thigh. The lesions were dry, raised, circular, scaling areas measuring up to 6 mm. in diameter. The patient's color was good although there was some respiratory distress. There were decreased vocal fremitus at the posterior right lung base and medium râles at both lung bases.

The patient was subjected to numerous laboratory tests, the majority of which yielded results within normal limits. However, the total proteins of the plasma were found to be 7.65 gm. per cent with a ratio of albumin to globulin of 3.27/4.38. The hemoglobin was 97 per cent with an increased resistance of the red blood cell fragility. There was a leukocytosis of 12,300, with a differential count of 492 staff forms, 6,519 polymorphonuclear cells, 123 mast cells, 4,305 lymphocytes, and 861 monocytes based upon an examination of 100 white blood cells. The corrected sedimentation velocity was 60 mm., and there was a reduced prothrombin concentration and delayed coagulation of the blood. Vitamin assays disclosed a normal amount of riboflavin in the urine and carotene in the blood; however, there was a markedly reduced content of vitamin A in the blood. The Wassermann and Kahn tests were negative. Biopsy of a skin lesion showed slight hypertrophy of the horny layer, some hemorrhage superficial to the stratum granulosum, scattered vesicle formation, slight parakeratosis, and a scanty infiltration of the corium with lymphocytes.

Roentgenograms of the chest taken on three different occasions disclosed a progressing peribronchial disease with some associated interstitial involvement; this appearance then gave place to one of progressive coalescence. A review of these films by Dr. C. B. Peirce, after the diagnosis of the condition was made at autopsy, failed to disclose any pathognomonic roentgenologic features.

The patient was treated with 2,070,000 units of penicillin over a total period of

17 days without appreciable effect, her temperature remaining at about 100° F. She was affected with increasingly severe dyspnea and cyanosis, and died in respiratory failure 41 days after admission and about 13 weeks after the onset of symptoms.

The clinical impression was of an unusual bronchopneumonia of unknown cause.

POST-MORTEM EXAMINATION

A complete necropsy was performed by Dr. B. A. Levitan 13 hours after death. The only pertinent findings were in the thorax and colon. The heart and pericardial cavity were normal. The pleural cavities contained a minimal amount of clear fluid. The parietal pleurae were smooth. A thin layer of fibrinous exudate covered the visceral pleura of the anterior aspect of the right lower lobe and the posterior and lateral aspects of the left lower lobe. The right lung weighed 800 gm., while the left weighed 1,000 gm. The lungs appeared to be distended and did not collapse. Upon palpation they were firm and noncrepitant except in the right upper lobe where a slight degree of crepitation was elicited. On section the cut surfaces were granular and slightly moist, varying from gray to a very light grayish white. All lobes were involved, the least involvement being in the right upper lobe which was very light grayish white. The larynx, trachea, and bronchi were normal. The peribronchial and mediastinal lymph nodes were not enlarged.

There were multiple intestinal polypi from the transverse colon to the rectum.

No other organs showed significant abnormalities. The salivary glands were not examined.

Bacteriologic Examination. From a swab from the lung a small number of *Streptococcus viridans* and *Escherichia coli* were grown. The heart blood contained some contaminant corynebacteria.

Microscopic Examination

The tissues were fixed in formol-saline solution and selected sections were stained with hematoxylin and eosin, Weigert's fibrin stain, Glynn's stain for bacteria, and Masson's trichrome stain.

The histopathologic findings in the thyroid, parathyroid, heart, liver, spleen, pancreas, jejunum, colon, kidneys, brain, and vertebral bone marrow were not pertinent. Both liver and adrenal glands showed the basophilic cytoplasmic granules described by Santee.[1] The salivary glands and other tissues were not sectioned.

Multiple sections from various parts of both lungs exhibited essentially similar features. The tissue preservation was only moderately good. The pleura was somewhat edematous and hyperemic. Occa-

sionally a minimal deposit of fibrin could be seen on its surface. Bronchi and bronchioles were filled with eosinophilic coagulum, neutrophilic polymorphonuclear cells, and a few large and small mononuclear cells. The epithelium of the bronchi and bronchioles had separated from its basement membrane and could be identified only as small isolated fragments. This epithelium varied from a cuboidal-columnar to a pseudostratified ciliated columnar form. No inclusion bodies were found in the cytoplasm or nuclei of these cells. The lung parenchyma, generally, was greatly altered. The alveoli were distended by large plugs of protein coagulum containing little stainable fibrin, or by masses of inflammatory cells consisting of polymorphonuclear leukocytes with a considerable admixture of plasma cells, large and small mononuclear cells, and swollen macrophages. Among the inflammatory cells, isolated polyhedral septal cells were seen. Many alveoli contained varying proportions of coagulum, fibrin, and inflammatory cells, while extravasated erythrocytes were present in moderate numbers. Atria and bronchioles were filled with similar material. The alveolar walls were widened by edema fluid and their capillary channels were engorged with blood but interstitial inflammatory mononuclear cells were found in relatively few areas. The cells lining the alveoli were large and prominent, varying from a low flattened cuboidal type with a spindle-shaped nucleus to a swollen polyhedral or slightly elongated cell with a round or oval vesicular nucleus. The cells often lost their identity as individual cells, apparently becoming syncytial, and there was a tendency for sheets of cells to separate from the alveolar walls. No definite inclusion bodies were found in these cells; however, they appeared to be transformed into cells that did contain such bodies.

The inclusion-bearing cells varied considerably in size and form, being found attached to the alveolar walls or, often, lying free in the alveolar spaces. They varied in number from one to several per high-power field. The earliest recognizable form was somewhat larger than the cells already described, measuring 14 μ in its major diameter. It usually lay attached to the alveolar wall between two of the swollen cells described above, and bulged further into the lumen than they did. The nucleus became metachromatic or acidophilic and, while not homogeneous, it was more dense and uniform than the vesicular nuclei previously mentioned. Sometimes there was the appearance of many discrete, round, acidophilic bodies and fragmented chromatin within the nucleus. The cytoplasm, too, showed vague, small, basophilic bodies within it and a slightly lacy texture. Cytoplasmic inclusions were not seen in the absence of evidence of nuclear inclusions.

The next stage of development of the inclusion body was more

easily recognized. The affected cells were usually large, being about 18 μ in size. A distinct round or oval inclusion body about 5 μ in length occupied the center of the nucleus. It was usually more acidophilic than metachromatic. Its edges were seldom sharply defined and it often had a honeycomb or conglomerate appearance. Surrounding this body was a halo of nuclear sap with low chromatic power, and surrounding this again was a sharply defined, distended nuclear membrane with a finely divided, marginated chromatin layer. Just within the nuclear membrane several discrete, minute, round or irregular basophilic bodies were seen. The nucleus tended to be somewhat eccentric. The cytoplasm usually contained definite and numerous round or oval basophilic bodies about 2 μ in diameter. These bodies were frequently less densely stained in their central portion than at their periphery. The largest cells seen averaged about 25 μ in size. They were irregular in shape and sometimes contained two or three nuclei that frequently showed different stages in the development of the contained inclusion bodies.

The most advanced forms encountered showed an ovoid body about 8 μ in length. It was homogeneous and markedly acidophilic but occasionally showed a metachromatic core. A very clear halo surrounded it, but due to the size of the inclusion, the achromatic space was reduced to a few μ in width. The nuclear membrane was again distinct and sharp but the number of small basophilic bodies seen within its margin was reduced. The cytoplasm of the largest cells contained as many as 20 or 30 small, round or oval basophilic bodies measuring 1 to 4 μ in diameter and tending to be localized somewhat in one area of the cytoplasm. Occasionally one of these bodies could be seen to indent the nuclear membrane.

Cytoplasmic and intranuclear inclusions similar to those described in the lungs were found in very small numbers in the epithelial cells of the zona reticularis of the adrenal cortex.

DISCUSSION

In 1925 VonGlahn and Pappenheimer [2] reviewed the literature concerning intranuclear inclusion bodies in tissues other than skin and central nervous system. To the 16 previous reports of such lesions in infants they added their own case of intranuclear inclusions in the intestine, liver, and lungs of a 36-year-old male. The description of the pulmonary lesions in their case bears a substantial resemblance to the findings reported here, but the authors did not emphasize cytoplasmic inclusions. The literature was again reviewed by Farber and Wolbach [3] in 1932. They assembled 25 cases, all except that of VonGlahn

and Pappenheimer being of infants. To these they added 26 new cases from a series of 183 consecutive autopsies on infants and children less than 1½ years of age. In these cases the inclusions were confined to the salivary glands in 24, and were found in various of the viscera, including the lungs, in 2. Their description and illustrations of the intranuclear and cytoplasmic inclusions in these 26 cases are identical in all respects with those found in the present case. The authors stated that the inclusion bodies were identical with the virus inclusion bodies of the salivary glands of guinea-pigs as reported by Pearson,[4] while the latter concluded that the viruses of the guinea-pig and of man were "very similar, if not one and the same."

Although inclusion disease of this type is not a rare lesion of the salivary glands, it is distinctly uncommon as a lesion of other epithelial viscera. In the report of a case of widespread inclusion disease in an infant, Kinney,[5] in 1942, stated that only 25 cases had been reported in which organs other than the salivary gland were involved and that in only 11 of these had the inclusions been found in several viscera. It is interesting to note that the disease is apparently quite asymptomatic unless the lungs are affected.

In addition to the adult case with pneumonitis reported by VonGlahn and Pappenheimer,[2] 7 other examples of inclusion disease in the adult have been found in the literature.[6-10] These included lesions in the stomach and esophagus, and an anal tumor. The inclusions were morphologically identical with those of the present case, with those of the disease in infants, and with those of virus diseases of the salivary glands in guinea-pigs.

Examination of the various reports of both infantile and adult inclusion disease discloses the occasional failure to find cytoplasmic inclusions, and offers positive evidence that inclusions may be found in various organs in cells of both epithelial and mesenchymal derivation. The failure to demonstrate cytoplasmic inclusions does not appear to invalidate the diagnosis of inclusion disease, for the large size of the nuclear inclusions is almost pathognomonic of the lesion. In the present case cytoplasmic inclusions never were found in the absence of evidence of nuclear inclusions. Indeed, if one may reason by analogy, the occasional absence of cytoplasmic inclusions may be expected, for animal experimentation [11] has shown that, in salivary gland disease in guinea-pigs, the formation of intranuclear inclusions precedes the development of cytoplasmic inclusions by 7 to 10 days.

While the lesions of inclusion disease are highly characteristic, there are various other conditions which will give rise to inclusion phenomena with or without giant cell formation. Diseases which give rise to both

cytoplasmic and nuclear inclusions are not numerous, comprising small-pox, paravaccinia, alastrim, Hecht's giant cell pneumonia, and distemper.[12] In all of these diseases the cytoplasmic inclusions are acidophilic and the nuclear inclusions are small. Inclusions that are induced by chemicals such as bismuth or aluminum hydroxide are usually intra-nuclear, small, and endowed with peculiar staining reactions, but they are occasionally seen in the cell cytoplasm in the same form in which they appear within the nucleus.[13-15] It is obvious that the basophilic cytoplasmic inclusions of inclusion disease are of diagnostic value.

The number of pulmonary diseases in which multinucleated giant cells and inclusion bodies may be found are few, comprising Hecht's giant cell pneumonia, animal distemper, and measles. Hecht's giant cell pneumonia and animal distemper exhibit in the lungs large, irregular, syncytial cells containing many nuclei and showing small acidophilic inclusions in both nuclei and cytoplasm.[12] The giant cells of measles, on the other hand, while approximating the morphologic characteristics of Hecht's giant cells, apparently show acidophilic cytoplasmic inclusions only.[16]

It is apparent that the single or binucleated giant cells of inclusion disease with their complement of very large, acidophilic nuclear inclusions and small, rather uniform, basophilic cytoplasmic inclusions constitute a highly characteristic morphologic entity. That the human disease is actually caused by a virus is not established. However, the general structure of the inclusion bodies is of a type that has been shown to be associated with viral agents in both man and experimental animals. Furthermore, the lesion is morphologically identical with a proved virus disease of the guinea-pig that has been shown to be capable of unusual virulence.[11]

It was indicated in the clinical summary of the present case that the patient suffered from a marked deficiency of vitamin A content of the blood and also developed a skin eruption that might suggest a nutritional deficiency. The coincidence of virus infection and vitamin A deficiency is not a new observation. Among the group of infants showing inclusion disease reported by Farber and Wolbach [3] there were two with the dermatologic manifestations of vitamin A deficiency. Chown [17] maintained that the pathologic changes in the lungs in giant cell pneumonia were a manifestation of vitamin A lack, and Pinkerton et al.[12] have pointed out that many of the clinical and pathologic features of distemper resemble those of such a deficiency. While the interpretation of such observations is beyond the scope of a simple morphologic report, it should be pointed out that the patient was given vitamin supplements when her deficiency was discovered, and that no

morphologic evidence of vitamin A privation was found at autopsy. Moreover, there was no apparent mitigation of the patient's clinical condition at any time during her 6 weeks' hospitalization.

SUMMARY

A case of fatal inclusion-disease pneumonitis in an adult is presented with a detailed study of its morphologic features and a comparison of the lesions with those of other relevant diseases that show inclusion phenomena. The coincidence of vitamin A deficiency and some viral diseases is mentioned. This constitutes the second case of inclusion-disease pneumonitis to be reported in an adult.

I am indebted to Drs. H. Pinkerton and Ph. H. Hartz, both of whom have examined sections from the lungs of this case and concur in the diagnosis.

REFERENCES

1. Santee, F. L. Peculiar granules in the cells of the liver and adrenal in infections. *Bull. Johns Hopkins Hosp.*, 1936, 59, 427–434.
2. VonGlahn, W. C., and Pappenheimer, A. M. Intranuclear inclusions in visceral disease. *Am. J. Path.*, 1925, 1, 445–466.
3. Farber, S., and Wolbach, S. B. Intranuclear and cytoplasmic inclusions ("protozoan-like bodies") in the salivary glands and other organs of infants. *Am. J. Path.*, 1932, 8, 123–135.
4. Pearson, E. F. Cytoplasmic inclusions produced by the submaxillary virus. *Am. J. Path.*, 1930, 6, 261–274.
5. Kinney, T. D. Intranuclear inclusions in infancy. *Am. J. Path.*, 1942, 18, 799–811.
6. Hartz, P. H. Protozoa-like cells in gastric ulcers. *Acta brev. Neerland*, 1935, 5, 115–117.
7. Hamperl, H. Beiträge zur normalen und pathologischen Histologie der Magenschleimhaut. *Virchows Arch. f. path. Anat.*, 1935–36, 296, 82–113.
8. Soeharto, R. Over zweren in maag en duodenum bij Inheemschen en Chineezen in Nederlandsch-Indië. [Dissertation.] Academisch Proefschrift Batavia. D. G. Kolff & Co., Batavia, 1937. (Cited by Hartz and van de Stadt.[10])
9. Hartz, P. H., and van der Sar, A. Over het voorkomen van de zoogenaamde protozoaire cellen in een ulcus oesophagi. *Geneesk. tijdschr. v. Nederl.-Indië*, 1941, 81, 1310–1313. (Cited by Hartz and van de Stadt.[10])
10. Hartz, P. H., and van de Stadt, F. R. The occurrence of protozoan-like cells in a biopsy from the anus. *Am. J. Clin. Path.*, 1943, 13, 148–153.
11. Rosenbusch, C. T., and Lucas, A. M. Studies on the pathogenicity and cytological reactions of the submaxillary gland virus of the guinea-pig. *Am. J. Path.*, 1939, 15, 303–338.
12. Pinkerton, H., Smiley, W. L., and Anderson, W. A. D. Giant cell pneumonia with inclusions. A lesion common to Hecht's disease, distemper and measles. *Am. J. Path.*, 1945, 21, 1–23.
13. Olitsky, P. K., and Harford, C. G. Intranuclear inclusion bodies in the tissue reactions produced by injections of certain foreign substances. *Am. J. Path.*, 1937, 13, 729–747.

14. Olitsky, P. K., and Harford, C. G. Further observations on intranuclear inclusions produced by non-virus materials. *Proc. Soc. Exper. Biol. & Med.*, 1938, 38, 92–94.

15. Pappenheimer, A. M., and Maechling, E. H. Inclusions in renal epithelial cells following the use of certain bismuth preparations. *Am. J. Path.*, 1934, 10, 577–588.

16. Corbett, E. U. The visceral lesions in measles. With a report of Koplik spots in the colon. *Am. J. Path.*, 1945, 21, 905–919.

17. Chown, B. Giant cell pneumonia of infancy as a manifestation of vitamin A deficiency. *Am. J. Dis. Child.*, 1939, 57, 489–505.

DESCRIPTION OF PLATE

PLATE 146

FIG. 1. Photomicrograph of lung. A low magnification shows hyperemic, swollen alveolar walls enclosing alveolar spaces distended by acidophilic coagulum and inflammatory cells. Numerous inclusion-bearing giant cells lie adjacent to alveolar walls. Masson's trichrome stain. $\times$ 130.

FIGS. 2 and 3. Photomicrographs of lung. Detail of the inclusion body giant cells is shown at high magnification. The relation of the cells to the alveolar wall is seen in Figure 2. Of note are the sharply demarcated nuclear membrane, the halo about the intranuclear inclusion, and the large size attained by the latter. Clumped cytoplasmic inclusions are well shown in Figure 2. Hematoxylin and eosin stain. $\times$ 860.

McMillan

Inclusion-Disease Pneumonitis in an Adult
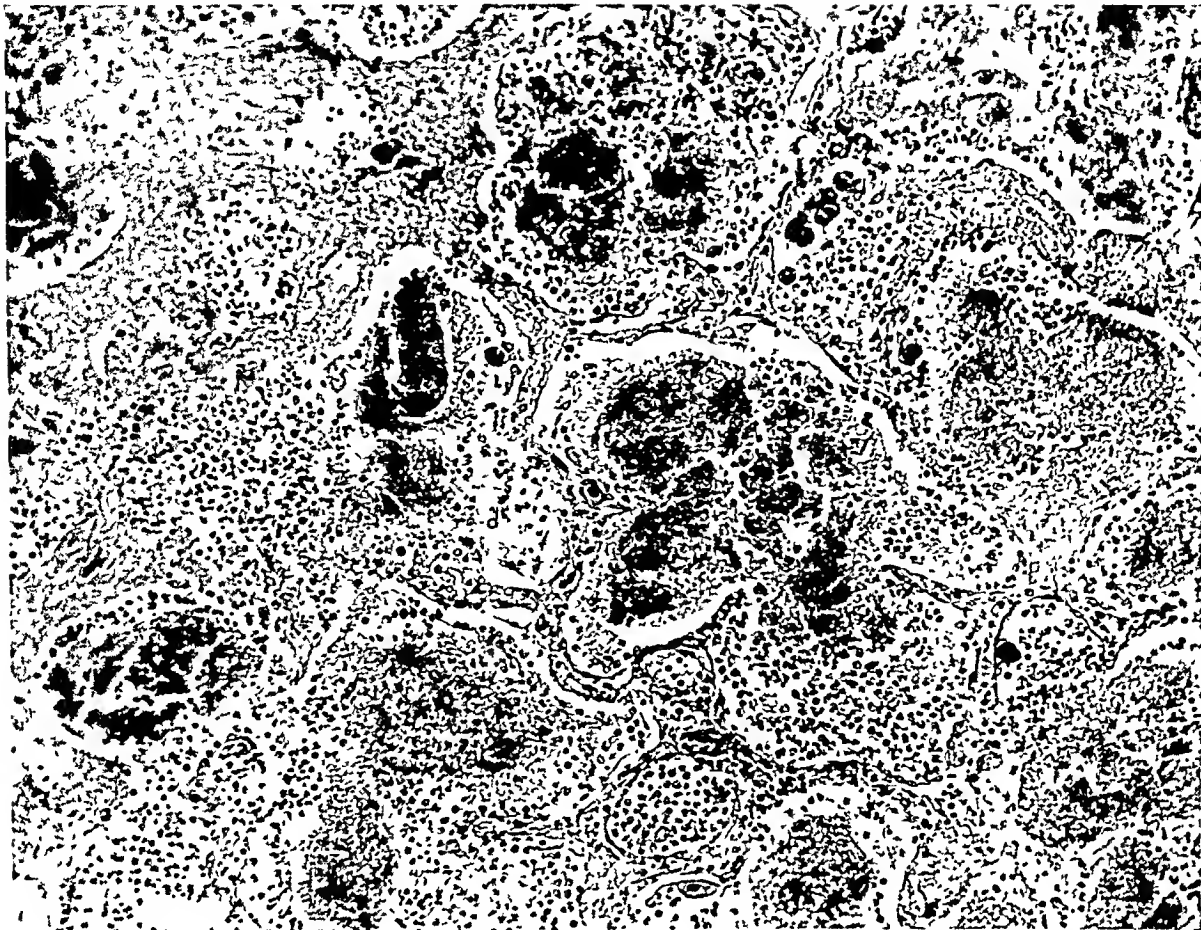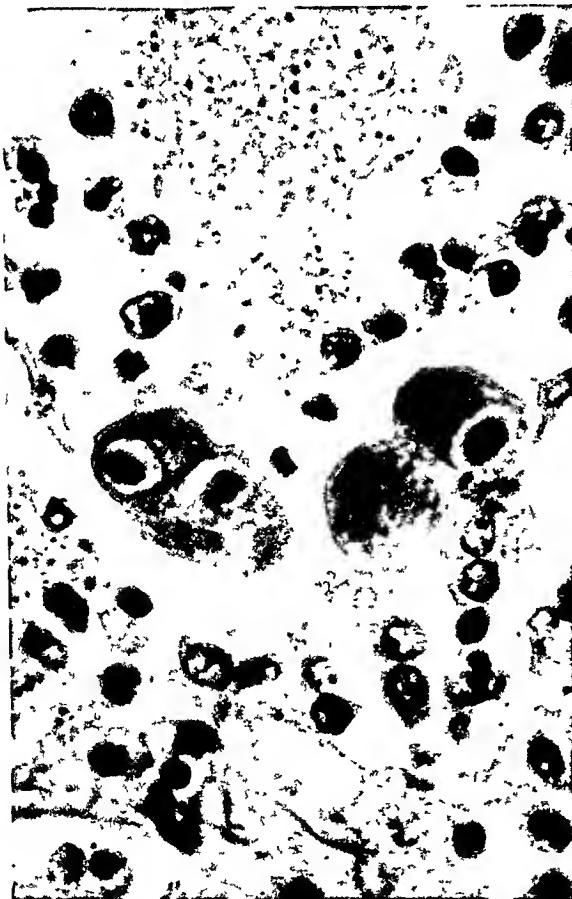
INTRANUCLEAR INCLUSIONS IN THE ISLANDS OF LANGERHANS OF CHICKENS *

ALFRED M. LUCAS, Ph.D.

(From the U.S. Regional Poultry Research Laboratory, East Lansing, Mich.)

During the course of investigations of cytologic problems in relation to lymphomatosis in chickens, intranuclear inclusions were found in the islands of Langerhans. Since lymphomatosis is the only common disease of chickens at this laboratory and since external and internal parasites are absent except for coccidia in a low percentage of birds,[1,2] there is justification for an attempt to correlate such widely divergent manifestations as inclusion bodies in the pancreatic islets and the occurrence of lymphomatous tumors in various regions of the body. Intranuclear inclusions often indicate the presence of a virus and it has been suggested that filtrates from visceral lymphoid tumors[3,4] and from tumor strains derived by serial passage from naturally occurring lymphomatosis will produce lymphomatous lesions.[5]

The present investigation has been limited to a study of the location and structure of inclusion bodies, the cycle of inclusion formation and degeneration, and the incidence of affected birds in relation to sex, age, and the presence of gross lesions of lymphomatosis.

MATERIALS AND METHODS

The chickens available at this laboratory were Single-Comb White Leghorns. The genetic history and performance of the stock are available in several publications.[1,2,6-8]

Examination was made of all suitably preserved cases from which a section of the pancreas had been saved. This material was from chickens of both sexes and of a wide range in age, from 15-day embryos to chickens 1,158 days old. At gross necropsy examination, many birds were negative, some were positive for naturally occurring lymphomatosis, and still more were positive for lymphomatous tumors following inoculation with different lymphomatous tumor strains.

Chickens from 6 different years (1940–1945) were represented, although most of the birds studied were hatched in 1942 to 1944 inclusive. In addition, 10 birds, 161 to 219 days of age, hatched in 1944, were available from the source stock of Iowa State College, which was used in experiments on lymphomatosis.

The fixatives most commonly used were 10 per cent formalin; Zenker's fluid with formalin or acetic acid; and Petrunkevitch's fluids nos.

1 and 2.[9] Of these fixatives, Petrunkevitch no. 2 was by far the best for showing intranuclear inclusions in the islands of Langerhans. In a few cases fixed in Carnoy's fluid or alcohol-acetic mixture, fair results were obtained. The stains used were Giemsa, MacNeal's tetrachrome, hematoxylin-azur II-eosin, and hematoxylin with eosin or triosin. The hematoxylin and triosin, after Petrunkevitch no. 2, gave excellent results; the inclusions were so brilliantly stained that they were easily identified with the 8 mm. objective. The inclusion bodies were brought out very clearly when material fixed in Zenker-acetic fluid was stained with malachite green and and acridine red.[10] The inclusion bodies are not artifacts of fixation or staining since they could be identified under high magnification with all technics mentioned. However, in many combinations such as formalin fixation followed by hematoxylin and eosin, they appeared pale and often colorless so that they easily could have been overlooked.

The diagnosis of "inclusions present" or "inclusions absent" was based on a single section. The few negative cases found among older birds might have been diagnosed as positive if additional sections had been cut and examined, but it seemed desirable to treat all material in the same way since it would have been impossible to determine positively the absence of inclusion bodies except by complete serial sectioning of every negative case. Every portion of a section was examined under the microscope. In some cases only one inclusion body was found for the entire section; in other cases they were abundant and could be seen instantly. Those slides in which no inclusion body was found were examined thoroughly a second time.

OBSERVATIONS

The intranuclear inclusions were highly tissue-specific in their distribution; they were found only in the islands of Langerhans, never in the pancreatic acini or duct cells, and never in tissues from other organs.

As the examination of slides proceeded, the impression was gained that the inclusion bodies were most commonly found in islets of medium size and that they were usually absent from the extremely large and extremely small islets. Likewise, they were found nearly always in nuclei of cells devoid of granules and rarely in those cells filled with conspicuous, yellow-stained granules. On material fixed in Zenker-acetic fluid and stained with malachite green and acridine red, which in the same slide was discovered to give good tinctorial differentiation of alpha and beta cell granules and also zymogen granules as well as inclusion bodies, it was concluded that the inclusion bodies were

present only in the beta cells of the islets and not in the alpha cells. It was impossible to follow the cell boundaries in the granule-poor cells in material fixed with Petrunkevitch no. 2, and for that reason all boundaries were not included in the drawings. In the alpha cells filled with granules, the cell walls were more definite.

No quantitative measurements were made on the proportion of islet tissue to acinar tissue, and from a survey of the literature it appears not to have been worked out for any avian species beyond the fact that the splenic lobe appears to contain the highest proportion.[11,12] It was observed in the present study that in both the relative amount and in size the islets were small in embryos and young chicks up to several weeks of age. In older chicks there appeared to be a relatively rapid increase both in size of islets and amount of islet tissue. In grown chickens the size of the islets was rather variable, which agrees with Bensley's studies [13] on the guinea-pig. The smallest islet as seen in a single section was composed of a few cells; the largest measured 363 by 458 μ. Cyst-like cavities sometimes were present in the large islets. The cavities were located on the side of the cell opposite the blood vessels. The largest lumen found was 23 μ in diameter. However, in many islets, the lumen, if present, was only a narrow slit. In mammals a lumen among islet cells has been said to be absent [14] and was not reported in the review by Opie,[15] although Clara described a small lumen among the cells of the islands of Langerhans in man [16] as well as in birds.[11] When the lumen was large, the nuclei of the marginal cells sometimes were closely adjacent to the cavity, but if the lumen was narrow and small there was usually no indication of cell polarity as shown by the position of the nucleus.

The acinar portion of the pancreas, in many cases, showed conspicuous lesions of various types. In many birds the acinar tissue was largely depleted of its zymogen granules and the cells and their nuclei were often distorted. Areas in which the cells appeared normally filled with zymogen granules were often multi-focal in distribution. Among the depleted cells many showed a large vacuolated cytoplasm; often the vacuole was large enough to deform the nucleus, and frequently it contained an acidophilic body surrounded by a clear halo. Against the wall of the vacuole was pressed the ergastoplasm or basophilic material of the cell. At first glance the whole configuration gave the suggestion of an intranuclear inclusion, but on closer examination it proved to be entirely cytoplasmic and probably was not an inclusion body of viral type. It was assumed that the acidophilic body inside the vacuole represented an abnormal clumping and degeneration of zymogen granules, or it may be the avian equivalent of Bensley's interpretation [13]

of the Mankowski granules in mammals, but additional data are needed to prove the validity of these suggestions.

In addition to the various cellular changes in the acinar cells, lymphoid accumulations of various sizes frequently occurred. They were usually focal and were located in the interacinar connective tissue or in the loose connective tissue near the larger vessels and ducts. With increased growth of the lymphoid tissue and especially with its extensive growth in tumor strains, both acinar and islet tissues were pushed aside or destroyed. In marked contrast was the complete freedom of the islands of Langerhans from any lymphoid tissue. It was expected that occasionally a few lymphocytes would be found around the small vessels in the islets, as in other regions of the body, but none were observed even when tumors were present in nearby regions of the pancreas.

Description of Inclusion Body and Nuclear Reaction

Typical, resting nuclei (Figs. 1 and 2) were round or oval and showed some variation in size. The chromatin was scattered along the linin fibers and part of it was adherent to the inner surface of the nuclear membrane. No distinct nucleolus was present but some nuclei showed a central clumping of chromatin around a colorless or pale, blue-staining, refractile body (Figs. 1 and 4) which possibly represented an amphinucleolus.

The intranuclear inclusions (Figs. 3 to 20) were strongly acidophilic after fixation with Petrunkevitch's no. 2, and were always homogeneous and never refractile, even when small. There was considerable range in size. Measurements made from the drawings, which had been outlined by means of a camera lucida, gave 0.37 μ for one of the bodies in Figure 4 and 4.15 μ for the large one in Figure 18. Measurements made with a filar micrometer, of cells other than those illustrated, showed a range in size from 0.4 to 4.0 μ, or a 1,000-fold increase in volume from the smaller to the larger.

Early stages in the development of inclusion bodies were considered to be those in which the nuclei showed a minimal amount of retraction of chromatin and linin network toward the membrane. This criterion had proved to be a reliable one in previous studies.[17-20] Initial stages in margination were extremely rare (Figs. 3 and 4). Even in the examples illustrated, margination was well underway and the resulting halo of nucleoplasm around the inclusion body was well developed. In the early stages the inclusion bodies themselves were single or multiple and showed considerable variation in size. Regardless of size, they were always homogeneous and never granular at any stage. Therefore,

it was concluded that these inclusion bodies are of the multiple homogeneous type, and that they are not like the inclusions of panleukopenia in cats,[20] which are granular in the first part of the cycle and become homogeneous in later stages.

In areas where inclusions were relatively abundant, adjacent non-inclusion-bearing nuclei were examined to find earlier stages than those represented in Figures 3 and 4 and to determine whether there might be an indication that the inclusion arose from the plasmosome portion of an amphinucleolus (if the light-staining sphere in the centrally placed clump of basichromatin in Figure 1 can be considered as an amphinucleolus). No transitional stages were found between this plasmosome-like body and the inclusion body. Even more convincing evidence against a plasmosome origin came from the fact that the amphinucleolus-like structure was marginated with the other chromatin (Fig. 4).

There still remained the possibility that the inclusion bodies arose from oxychromatin, but again no transitional stages were observed, although small clumps of oxychromatin were sometimes found in non-inclusion-bearing nuclei. Thus, they were different from the homogeneous inclusions of fox encephalitis in which the acidophilic portion of the inclusion body is derived from oxychromatin.[18]

The next stage after the early reaction was the intermediate phase of the cycle. Practically all of the inclusions in the slides examined were in that phase (Figs. 5 to 18). Some still showed incompletely marginated chromatin and in others all of the chromatin had reached the nuclear membrane. There appeared to be no correlation between size or number of inclusion bodies and the stage of nuclear margination. Therefore, it was impossible to determine whether inclusions (Figs. 5 to 8) might sometimes grow by intussusception of material. However, there was good evidence that they do grow by accretion and coalescence; nuclei such as are shown in Figures 9, 11, 14, 15, and 16 seemed to indicate that the smaller bodies may arrange themselves in the form of a sphere and become merged into a larger body. Sometimes a small sphere was found which lay adjacent to a large one (Figs. 17 and 18) and the form of the inclusion body in Figure 19 seemed to indicate that such a small sphere could merge into a larger one. On the other hand, there seemed to be a certain amount of resistance to fusion of multiple spheres, even when large and touching each other (Fig. 10), and, likewise, when the spheres were small they sometimes showed little inclination to fuse (Figs. 12 and 13) but seemed to be scattered through the nucleoplasm at random.

Late stages in the cycle of nuclear reaction were as hard to find as

the early stages. Only a few nuclei were observed (Fig. 19) in which the marginated chromatin was pulled together in clumps characteristic of karyorrhexis, thus exposing bare, unstained sections of the nuclear membrane. A similar reaction was observed in late stages of fox encephalitis[18] and after subcutaneous inoculations of aluminum oxide.[19]

One nucleus (Fig. 20) was found in which the nuclear membrane appeared to be broken and the inclusion body partly extruded. Rupture of the membrane apparently had caused lysis of some of the chromatin, giving the laked, homogeneous, pale-blue-staining material shown between the nuclear membrane and the inclusion body. (The ring-shaped body near the lower part of the nucleus was considered to be only a circle of chromatin.) It was thought that rupture of the nuclear membrane occurred while the tissue was alive and was not due to the drag of the microtome knife. In the latter case, there would have been no lysis of chromatin. One example is not adequate to determine the final stage in the life of the nucleus, but because the inclusion body seemed to be extruded a search was made for inclusion bodies in the cytoplasm of islet cells. The evidence for the existence of extranuclear inclusions thus far has not been sufficiently definite to be conclusive.

The possibility was tested that the inclusion bodies might contain glycogen since Chipps and Duff[21] reported that empty or ghost nuclei of the liver contained large glycogen spheres which were dissolved in the usual technics. Particularly significant was the fact that 23 per cent of the cases showing glycogen inclusions were from patients with uncontrolled diabetes mellitus. Chicken pancreas was fixed in Carnoy's fluid or in acetic acid-alcohol and stained with Best's carmine. The large inclusions in the islets were negative for glycogen and the small ones sometimes took a faint pink coloration with the stain, which, however, was not sufficiently strong to be regarded as positive for glycogen.

Relation of the Incidence of Inclusion Bodies to Age

The data in Table I show the presence or absence of inclusion bodies of the islets in birds of different ages, the number of each sex involved, and the presence or absence of gross lesions of naturally occurring lymphomatosis or its tumor strains.

Since the cases reported represented a variety of experiments and projects, the distribution of birds at different ages is not uniform; for example, tissues from normal young birds were lacking. Yet, with the

cases available, the results show a definite relationship between age and the presence or absence of inclusions. No inclusion bodies were found in any of 39 birds, from 15-day embryos to chicks 30 days old. Inclusion bodies were present in 230 of 235 birds, or 97.9 per cent, above 30 days of age. When these were broken down into smaller age groups, 75 per cent (6 of 8) were positive at 31 to 40 days of age, 93.7 per cent (15 of 16) were positive at 41 to 50 days of age, 97.7 per cent

TABLE I

Incidence of Intranuclear Inclusions in the Islands of Langerhans in Relation to Lymphomatosis, Age, and Sex

Age in days	No. of cases	Inclusions present				Inclusions absent			
		Lymphomatosis		Negative for lymphomatosis	Total	Lymphomatosis		Negative for lymphomatosis	Total
		Tumor strains	Naturally occurring disease			Tumor strains	Naturally occurring disease		
Embryos									
15–21	7							7	7
After hatching									
1–10	12					2	3(?)	7	12
11–20	8					8			8
21–30	12					12			12
31–40	8	6			6	2			2
41–50	16	15			15	1			1
51–100	43	35	6	1	42	1			1
101–300	53	6	8	39	53				
301–500	42		1	41	42				
501–1000	71		6	64	70			1	1
1001–1158	2			2	2				
Totals	274				230				44
Males					68				23
Females					162				18
Sex not determined									3
Totals					230				44

(42 of 43) were positive at 51 to 100 days of age, and 99.4 per cent (167 of 168) were positive at 101 to 1,158 days of age. Thus, the critical age in the transition from the absence of inclusion bodies to their presence was 30 days as based on 10 day age groups.

Ten birds, 161 to 219 days of age (3 males and 7 females), from the source stock of Iowa State College for studies on lymphomatosis, all showed intranuclear inclusions in the islands of Langerhans.

Relation of Inclusion Bodies to Sex

The utilization of a small number of males in breeding pens resulted in a low proportion of males to females in the older birds, but from the

cases available it was found that the presence of inclusions was not associated with one sex more than with the other. As shown in Table I, 23 males and 18 females showed no inclusion bodies, while inclusion bodies were found in 68 males and 162 females.

Relation of Incidence of Inclusion Bodies to Incidence of Lymphomatosis

Those who have studied lymphomatosis usually have not included in their data birds which died before 30 days of age [1] or even before 160 days of age.[22] The 3 cases listed under "naturally occurring disease" in the age group 1 to 10 days (Table I) were questionable cases of neural and visceral lymphomatosis. Birds inoculated with cellular or cell-free filtrates of lymphomatous tumor strains were in the age range of 10 to 171 days, and it was from this material, which was well distributed both before and after 30 days of age, that it was concluded that no relationship exists between the tumor strains and inclusion bodies in the pancreas. The older birds were nearly all from a line which is susceptible to lymphomatosis but due to isolation had shown only a low incidence of the disease. The number of grossly negative birds was large compared with the number of grossly positive birds, but many of the grossly negative birds were microscopically positive. On the basis of gross lesions, there appeared to be no relationship between naturally occurring lymphomatosis and the development of inclusion bodies in the islands of Langerhans. However, no tests have been devised for recognition of the etiologic agent, or agents, of lymphomatosis other than by the presence of the lymphoid tumors. Thus, it is at least theoretically possible that many or even all birds were carrying the disease agent.

The birds listed in Table I were from inbred lines,[1,2] as follows:

Selected for Resistance		Selected for Susceptibility	
Line	No. of birds	Line	No. of birds
5	1	2	8
10	1	7	23
		9	2
		11	2
		14	3
		15	231

It is obvious from these data that susceptible line 15 is the only one adequately represented. It might prove to be true later that other lines have a different incidence and age relationship than are shown by the data thus far obtained. The one bird over 500 days of age which was negative for inclusions came from line 2.

DISCUSSION

The data thus far obtained have given the following information: Intranuclear inclusions occurred in the cells of the islands of Langerhans of the White Leghorn chickens studied in nearly all birds over 30 days of age. The structure and location of the inclusion bodies and their cycle of development are in harmony with the idea that a virus might be present. The only disease, except coccidiosis, known to exist in the birds used was lymphomatosis, and there is evidence in the literature [3-5] that visceral lymphoid tumors, both naturally occurring and in a tumor strain, carry a filtrable agent or agents. Yet there was no evidence of correlation between lymphomatosis when based on gross diagnosis and the presence of inclusion bodies in the pancreas.

If the inclusion bodies do signify the presence of a virus, it must be latent, since inclusions are present in some birds which to all appearances are normal and healthy. Andrewes [23] and Oberling [24] have discussed numerous examples of latent viruses. The submaxillary gland virus is also a good example and a review of the literature concerning this virus permits some suggestive analogies with the conditions found in the islets. Surveys have indicated that in one laboratory in England, 32 per cent (6 of 19) of the guinea-pigs were infected with the submaxillary gland virus.[25] In the New York area, 84 per cent of 75 guinea-pigs over 6 months of age carried the virus, and only 3 of 43 under 1 month of age showed inclusion bodies.[26] Thompson [27] found that 14 per cent (10 of 70) of the rats in a laboratory colony were infected and Rector [28] reported that 20 per cent (24 of 120) of wild rats carried inclusions of the submaxillary gland virus. Rector and Rector [29] reported that all of 14 ground moles were infected. Kuttner and Wang [30] observed that nearly all full grown Chinese hamsters carried the submaxillary gland virus. Cowdry and Scott [31] found salivary inclusions in 2 *Cebus fatuellus* monkeys and failed to find them in 18 *Macacus rhesus* monkeys.

There seems to be complete compatibility between the submaxillary gland virus and the host except for slight monocytic infiltrations of the gland. Yet when the infected gland from guinea-pigs is injected intracerebrally into noninfected animals, it causes death in 3 to 16 days.[17,25,26] The natural means of transmission is not known, but Cole and Kuttner [26] found that animals less than 30 days of age were reasonably certain to be free from the disease and this was confirmed by Scott [32] and by Pearson.[33] This is the same age range in which the inclusion bodies are absent in the pancreatic islets of chickens.

The inclusion bodies of the submaxillary gland virus differ in several

ways from those found in the pancreas of chickens. Although both belong to the homogeneous type, the inclusion body of the submaxillary gland virus is usually single and there are never more than two in a nucleus. The nucleus hypertrophies greatly, the chromatin returns from its marginated position on the nuclear membrane to apply itself on the inclusion body and, finally, a cytoplasmic inclusion is present in addition to the one in the nucleus.[17] The inclusions found in the islets are often multiple; there is only slight and often no hypertrophy of the nucleus; chromatin or linin fibers have never been found attached to the inclusion body; and cytoplasmic inclusions are absent. In an examination now under way of pancreases from wild birds, chromatin and linin fibers have been found attached to the inclusion body with a resulting retardation of margination. Multiple intranuclear inclusions of homogeneous type have been reported in the kidney of the Ceropsis goose,[34] and were found in the human kidney after lead poisoning[35] and after subcutaneous injections of aluminum oxide.[19]

In both of the latter examples of inclusions, which represent reactions to metals, considerable chromatin was attached to the inclusion bodies, and in the case of aluminum oxide the inclusion body was shown to arise from the plasmosome portion of an amphinucleolus. In the pancreatic islets of chickens there was no indication of adherent chromatin or of a plasmosome or oxychromatic origin, but since the early stages were few in number, plasmosome origin still remains a possibility.

If the inclusion bodies are not associated with a virus, then it is possible that they have some, as yet unknown, connection with physiologic activities in the islet tissue. An analogous example might be illustrated by the presence of intranuclear crystals in the hepatic cells of the Canidae which, through the work of Weatherford and Trimble,[36] appear to be related to the metabolism of purine bases, particularly uric acid excretion. The Dalmatian showed a lower number of crystal-bearing nuclei per animal than other breeds.[36,37] Crystals were found in 15 of 16 dogs examined. Cowdry and Scott[38] found the crystals in 22 per cent of 68 dogs examined. Weatherford and Trimble[36] concluded that neither age nor sex was a factor in the occurrence of intranuclear crystals. Intranuclear crystalloids have been reported by Dawson[39] in the superficial epidermal cells of the catfish, but no studies as to their cause have yet been made. He suggested that they might be related to degeneration of the cells.

An examination was made of the literature on the growth of the pancreas in birds, on carbohydrate metabolism, blood-sugar levels, and reactions to insulin. The percentile growth curve of the pancreas in

relation to body weight, according to Latimer,[40] showed at first a rise from 0.52 to 0.62 per cent. The latter maximum value was attained when the birds weighed 160 gm. Then followed a gradual fall to 0.20 per cent. The peak at 160 gm., according to Latimer's weight-age curve, fell at 30 days, which from the present study appears to be the critical age in the transition from absence to presence of inclusions.

Only a small amount of data was found in the literature on blood-sugar levels for chickens at different ages. In the embryo [41] it was as high as reported for the adult chicken.[42,43] Heller and Pursell [44] followed blood-sugar levels in hens from 1 to 27 months of age and observed a slight but not significant decrease with age. Batt [45] used chickens 6 months to 4 years of age and stated that "Immature birds tend to have a higher fasting blood-sugar level than adults." Golden and Long [46] found no significant difference in blood-sugar levels of birds weighing less than 300 gm. and those weighing more than 300 gm. In the common pigeon [47] there was a slight rise between the first 33 days and the next period from 57 to 66 days, but Scragglies and Ring Doves showed no differences at approximately the same age periods. Whether pigeons contain intranuclear inclusions in the islands of Langerhans similar to those found in the chicken is not yet known.

A virus disease of pigeons designated as due to an intranuclear inclusion agent has been reported. The intranuclear inclusions are found in both acinar and islet tissues of the pancreas as well as in liver and spleen.[48] This intranuclear inclusion disease was first found associated with psittacosis but was later isolated and the agent proved to be a virus smaller than psittacotic elementary bodies and to have a much greater host specificity.[49] The inclusions of intranuclear inclusion disease are reported to resemble those of herpes. Herpes produces a granular type of inclusion body but in the early stages may show homogeneous masses associated with the granules.[20] Thus it differs greatly from the inclusions found in the pancreatic islets of the chicken, which are homogeneous and multiple.

A report by Jungherr and Lucas [50] on intranuclear inclusions in the spleen of a poult is in process of preparation. Both homogeneous and granular types of inclusions were found but none belonged to the multiple homogeneous type found in the pancreas of the chicken, nor did the inclusions in the two species follow similar cycles of development.

Physiologic conditions as well as viruses can be responsible for intranuclear inclusions. In the survey of literature already presented it has been brought out that the inclusions in the islets of the chicken have certain features in common with either possibility. On the one

hand, the islet inclusions show a similar age relationship to those found in the viral submaxillary gland disease of guinea-pigs and the same high degree of compatibility with the host. In chickens as in guinea-pigs and ground moles there is a high incidence. On the other hand, a purely physiologic relationship, as seems to be the case in the crystals found in hepatic nuclei of dogs, is at present only a theoretical possibility in chickens because the physiologic data which might lead to correlations between structure and function are lacking. However, like dogs, chickens show a high incidence of inclusions in the populations studied and in both species there are no apparent pathologic lesions directly associated with the inclusion bodies.

It is evident, therefore, from the information and data thus far available that there is needed a study of the pancreas from numerous breeds of chickens and from various sources, as well as knowledge of the presence of similar inclusion bodies in species of birds which apparently do not carry the agent of lymphomatosis. Transmission studies are needed to determine whether a virus is present. The questions remain, also: Is the presence of intranuclear inclusions damaging to the normal function of the islands of Langerhans, and is the normal inter-relationship of the islets to hormone actions of adrenal, pituitary, and sex endocrines adversely affected?

SUMMARY

1. Intranuclear inclusions of the multiple homogeneous type were found in the islands of Langerhans of the White Leghorn chicken.

2. The cycle of development and degeneration of the inclusions was followed. The number of inclusions found within a nucleus varied from one to over a dozen. They ranged in size from 0.4 to 4.0 μ. Growth took place in some cases by coalescence of small inclusions to form larger ones, and growth by intussusception might have occurred also. In still other cases they remained multiple in character. Early stages were rare; in most nuclei, margination had proceeded far enough to give a well defined halo. Chromatin or linin fibers were never found attached to the inclusion body. Cytoplasmic inclusions were absent.

3. The presence of these intranuclear inclusions was related to the age of the bird. None was present under 30 days of age. In chickens 31 to 40 days of age they were present in 75 per cent of the birds; from 41 to 50 days of age, 93.7 per cent were positive; from 51 to 100 days of age, 97.7 per cent were positive; and from 101 to 1,158 days of age, 99.4 per cent contained inclusion bodies.

4. Sex seemed to have no influence on the incidence of the inclusions.

5. There was no correlation between the presence of inclusion bodies in the pancreatic islets and lymphomatous lesions as identified by gross examination only.

6. No definite significance of the inclusions in respect to latent virus diseases, growth, and physiologic factors can be assigned at the present time.

Since completion of the manuscript it has been brought to my attention that similar intranuclear inclusions of pancreatic islets have been observed by Jungherr and Levine.[51] In their study of the pathology of pullet disease they suggested that "Pathologically the condition is characterized by . . . . enlargement and apparent injury of the pancreatic islands." Other lesions associated with pullet disease were included besides the one mentioned. It must be concluded from studies on chickens at this laboratory, in which there has never developed a case of pullet disease, that the intranuclear inclusions of the pancreatic islets have no association with this disease.

REFERENCES

1. Waters, N. F. Breeding for resistance and susceptibility to avian lymphomatosis. *Poultry Sc.,* 1945, 24, 259–269.

2. Waters, N. F., and Prickett, C. O. Types of lymphomatosis among different inbred lines of chickens. *Poultry Sc.,* 1946, 25, 501–508.

3. Lee, C. D., Wilcke, H. L., Murray, C., and Henderson, E. W. Fowl leukosis. *J. Am. Vet. M. A.,* 1937, 91, 146–162.

4. Lee, C. D., and Wilcke, H. L. The migration of the etiologic agent of fowl leukosis when subjected to electrophoresis. *J. Am. Vet. M. A.,* 1939, 94, 178–186.

5. Burmester, B. R., Prickett, C. O., and Belding, T. C. A filtrable agent producing lymphoid tumors and osteopetrosis in chickens. *Cancer Research,* 1946, 6, 189–196.

6. Waters, N. F. The influence of inbreeding on egg weight. *Poultry Sc.,* 1945, 24, 318–323.

7. Waters, N. F. The influence of inbreeding on hatchability. *Poultry Sc.,* 1945, 24, 329–334.

8. Waters, N. F. The influence of inbreeding on sexual maturity. *Poultry Sc.,* 1945, 24, 391–395.

9. Petrunkevitch, A. New fixing fluids for general purposes. *Science,* 1933, 77, 117–118.

10. Hitchcock, C. H., and Ehrich, W. A new method for differential staining of plasma cells and of other basophilic cells. *Arch. Path.,* 1930, 9, 625–630.

11. Clara, M. Das Pankreas der Vögel. *Anat. Anz.,* 1923–24, 57, 257–265.

12. Nagelschmidt, L. Untersuchungen über die Langerhansschen Inseln der Bauchspeicheldrüse bei den Vögeln. *Jahrbuch f. Morphol. u. mikr. Anat.,* 1939, Abt. 2, 45, 200–232.

13. Bensley, R. R. Studies on the pancreas of the guinea-pig. *Am. J. Anat.,* 1911–12, 12, 297–388.

14. Bensley, R. R. Structure and relationships of the islets of Langerhans. Criteria of histological control in experiments on the pancreas. *Harvey Lectures,* 1914–15, 10, 250–289.

15. Opie, E. L. Cytology of the Pancreas. In: Cowdry, E. V. Special Cytology. Paul B. Hoeber, Inc., New York, ed. 2, 1932, 1, 375–405.

16. Clara, M. Eine Studie zur Kenntnis der Langerhansschen Inseln. *Ztschr. f. mikr.-anat. Forsch.*, 1924, Abt. 2, 1, 513–562.

17. Rosenbusch, C. T., and Lucas, A. M. Studies on the pathogenicity and cytological reactions of the submaxillary gland virus of the guinea-pig. *Am. J. Path.*, 1939, 15, 303–338.

18. Lucas, A. M. The cytology of fox encephalitis and the effects of centrifugation upon the intranuclear inclusions. *Am. J. Path.*, 1940, 16, 739–760.

19. Birch, F. M., and Lucas, A. M. Effects of centrifugation on intranuclear inclusions produced by subcutaneous injections of aluminum oxide. *Am. J. Path.*, 1942, 18, 1051–1060.

20. Lucas, A. M., and Riser, W. H. Intranuclear inclusions in panleukopenia of cats. A correlation with the pathogenesis of the disease and comparison with inclusions of herpes, B-virus, yellow fever, and burns. *Am. J. Path.*, 1945, 21, 435–465.

21. Chipps, H. D., and Duff, G. L. Glycogen infiltration of the liver cell nuclei. *Am. J. Path.*, 1942, 18, 645–659.

22. Hutt, F. B., Cole, R. K., Ball, M., Bruckner, J. H., and Ball, R. F. A relation between environment to two weeks of age and mortality from lymphomatosis in adult fowls. *Poultry Sc.*, 1944, 23, 396–404.

23. Andrewes, C. H. Latent virus infections and their possible relevance to the cancer problem. *Proc. Roy. Soc. Med.*, 1939–40, 33, 75–86.

24. Oberling, C. The Riddle of Cancer. (Tr. by W. H. Woglom.) Yale University Press, New Haven, 1944, 196 pp.

25. Andrewes, C. H. Immunity to the salivary virus of guinea-pigs studied in the living animal, and in tissue-culture. *Brit. J. Exper. Path.*, 1930, 11, 23–34.

26. Cole, R., and Kuttner, A. G. A filterable virus present in the submaxillary glands of guinea pigs. *J. Exper. Med.*, 1926, 44, 855–873.

27. Thompson, M. J. Intranuclear inclusions in the submaxillary gland of the rat. *J. Infect. Dis.*, 1932, 50, 162–170.

28. Rector, L. E. Coexistence of nuclear inclusions in salivary glands and kidneys of wild rats. *Proc. Soc. Exper. Biol. & Med.*, 1936, 34, 700–702.

29. Rector, E. J., and Rector, L. E. Intranuclear inclusions in the salivary glands of moles. *Am. J. Path.*, 1934, 10, 629–636.

30. Kuttner, A. G., and Wang, S. H. The problem of the significance of the inclusion bodies found in the salivary glands of infants, and the occurrence of inclusion bodies in the submaxillary glands of hamsters, white mice, and wild rats (Peiping). *J. Exper. Med.*, 1934, 60, 773–791.

31. Cowdry, E. V., and Scott, G. H. Nuclear inclusions suggestive of virus action in the salivary glands of the monkey, *Cebus fatuellus. Am. J. Path*, 1935, 11, 647–657.

32. Scott, G. H. Studies on the submaxillary virus of guinea-pigs. I. The effect of duct ligation and pilocarpine administration upon the cellular response to the virus. *J. Exper. Med.*, 1929, 49, 229–236.

33. Pearson, E. F. Cytoplasmic inclusions produced by the submaxillary virus. *Am. J. Path.*, 1930, 6, 261–274.

34. Cowdry, E. V., Lucas, A. M., and Fox, H. Distribution of nuclear inclusions in wild animals. *Am. J. Path.*, 1935, 11, 237–252.

35. Blackman, S. S., Jr. Intranuclear inclusion bodies in the kidney and liver caused by lead poisoning. *Bull. Johns Hopkins Hosp.*, 1936, 58, 384–402.

36. Weatherford, H. L., and Trimble, H. C. A further morphological and biochemical study on the intranuclear crystals in the hepatic cells of the dog—the pure breed and the hybrid Dalmatian. *Anat. Rec.*, 1940, 77, 487–507.

37. Weatherford, H. L. A morphological and experimental study of the intranuclear crystals in the hepatic cells of the dog. *Anat. Rec.*, 1938, 71, 413–445.

38. Cowdry, E. V., and Scott, G. H. A comparison of certain intranuclear inclusions found in the livers of dogs without history of infection with intranuclear inclusions characteristic of the action of filtrable viruses. *Arch. Path.*, 1930, 9, 1184–1196.

39. Dawson, A. B. Intranuclear crystalloids in the superficial epidermal cells of the catfish, *Ameiurus nebulosus. Anat. Rec.*, 1933, 56, 255–259.

40. Latimer, H. B. Postnatal growth of the body, systems, and organs of the single-comb White Leghorn chicken. *J. Agr. Research*, 1924, 29, 363–397.

41. Hanan, E. B. Experimental hypoglycemia and hyperglycemia in the chick embryo. *Proc. Soc. Exper. Biol. & Med.*, 1924–25, 22, 501–504.

42. Saito, S., and Katsuyama, K. Ueber den Zucker im normalen Hühnerblute. *Ztschr. f. physiol. Chem.*, 1901, 32, 231–234.

43. Koppányi, T., Ivy, A. C., Tatum, A. L., and Jung, F. T. Studies in avian diabetes and glycosuria. *Am. J. Physiol.*, 1926, 78, 666–674.

44. Heller, V. G., and Pursell, L. Chemical composition of the blood of the hen during its life cycle. *J. Biol. Chem.*, 1937, 118, 549–553.

45. Batt, H. T. The blood sugar level of the fasting domestic fowl. *Am. J. Physiol.*, 1939, 126, P429.

46. Golden, W. R. C., and Long, C. N. H. The influence of certain hormones on the carbohydrate levels of the chick. *Endocrinology*, 1942, 30, 675–686.

47. Riddle, O., and Honeywell, H. E. Studies on the physiology of reproduction in birds. XVI. The normal blood sugar of pigeons and its relation to age, sex, species and certain diseases. *Am. J. Physiol.*, 1923–24, 67, 317–332.

48. Smadel, J. E., Wall, M. J., and Gregg, A. An outbreak of psittacosis in pigeons, involving the production of inclusion bodies, and transfer of the disease to man. *J. Exper. Med.*, 1943, 78, 189–204.

49. Smadel, J. E., Jackson, E. B., and Harman, J. W. A new virus disease of pigeons. I. Recovery of the virus. *J. Exper. Med.*, 1945, 81, 385–398.

50. Jungherr, E., and Lucas, A. M. Intranuclear inclusions in a poult. (In preparation.)

51. Jungherr, E., and Levine, J. M. The pathology of so-called pullet disease. *Am. J. Vet. Research*, 1941, 2, 261–271.

[*Illustrations follow*]

DESCRIPTION OF PLATE

Drawings were made at an optical magnification of 1800 × and traced with a camera lucida at a projected magnification of 4076 ×.

PLATE 147

All nuclei are from beta cells of the islands of Langerhans. The tissues were fixed in Petrunkevitch no. 2 and stained with hematoxylin and triosin.

FIG. 1. A normal, noninclusion-bearing nucleus. The central, dark-stained chromatin mass enclosing a lighter stained sphere may represent an amphinucleolus.

FIG. 2. A normal noninclusion-bearing nucleus with diffusely scattered chromatin.

FIGS. 3 and 4. Early stages in the formation of inclusion bodies.

FIG. 3. The diffuse chromatin network beginning to marginate.

FIG. 4. Two inclusions, each measuring 0.37 μ in diameter. The amphinucleolus-like structure marginates with the chromatin and takes no part in the production of the inclusion body.

FIGS. 5 to 18. Intermediate stages of nuclear reaction.

FIGS. 5 to 7. Nuclei in which the chromatin has not fully marginated but the inclusions have grown to considerable size.

FIGS. 8 and 9. Similar stage of nuclear reaction as shown in Figures 5 to 7, but the inclusion bodies are multiple.

FIG. 10. A completely marginated nucleus which contains three large inclusions of equal size.

FIG. 11. A cluster of inclusion bodies in a partly marginated nucleus.

FIGS. 12 and 13. Multiple inclusions scattered at random with no tendency to aggregate in the form of a hollow sphere.

FIGS. 14 and 15. Multiple inclusions arranged in the form of a sphere. It is not certain whether the central homogeneous area in Figure 15 represents a larger inclusion body or whether it is merely the optical effect of the peripheral bodies at higher and lower levels.

FIG. 16. Peripherally placed inclusions fusing with the central inclusion mass.

FIG. 17. A lobulation of the nucleus somewhat like Figure 8. This nucleus contains two additional inclusions not shown, one at a low level of focus and one in the upper nuclear lobe.

FIG. 18. Extreme inequality of size of inclusion bodies in the same nucleus. The larger of the two measures 4.15 μ in diameter.

FIG. 19. A late stage in nuclear reaction in that the marginated chromatin is pulling together in clumps (karyorrhexis) leaving bare the unstained nuclear membrane. From the shape of the inclusion body it seems evident that a smaller inclusion is fusing with a larger one.

FIG. 20. A late stage showing a rupture of the nuclear membrane with liberation of the inclusion and laking (karyolysis) of the chromatin.
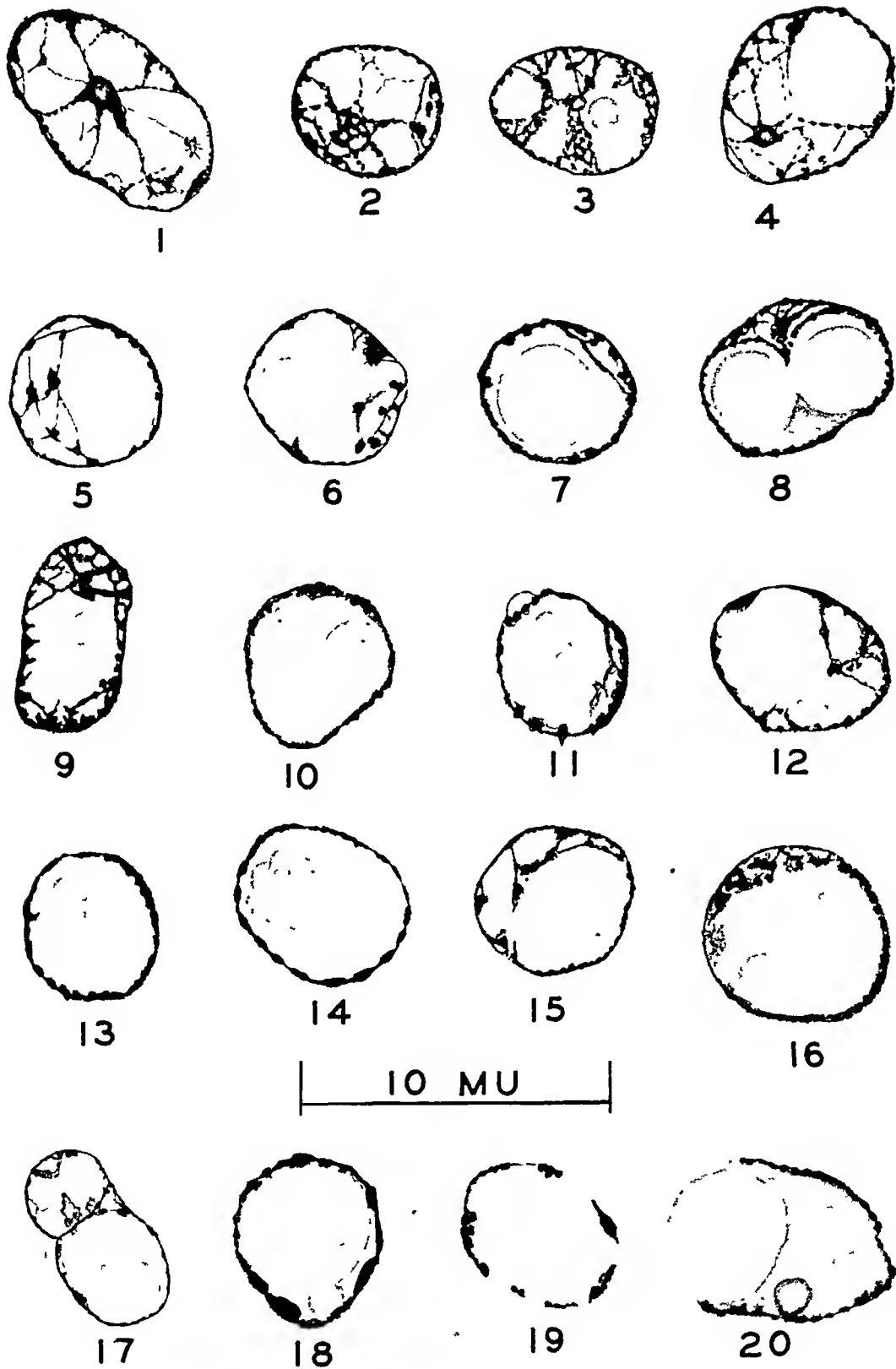
THE FATE OF ENDOCARDIAL VEGETATIONS FOLLOWING PENICILLIN TREATMENT OF BACTERIAL ENDOCARDITIS *

ARTHUR J. GEIGER, M.D., and STANLEY H. DURLACHER, M.D.
(From the Departments of Internal Medicine and of Pathology, Yale University School of Medicine, New Haven, Conn.)

The apparent curability of both acute and subacute bacterial endocarditis with penicillin has recently become well established clinically. Several clinics, including our own, have reported groups of cases in which such treatment has been followed by the disappearance of all symptoms and signs of the infection and sterilization of the blood for periods now reaching 3½ years. Such enduring remissions in a disease that usually runs its course to death within 1 year speak convincingly of cure. The argument that these cases may not be healed and merely represent examples of the rare or hypothetical "bacteria-free" stage of the disease is emphatically refuted by the considerable number of clinically cured cases already reported within the short period of 2 years. However, the complete and ultimate proof of cure rests on the demonstration of unequivocally healed and sterile remnants of vegetations in previously treated cases following death from other causes. This report presents four such cases.

PRESENTATION OF CASES

Case 1

A. G., a man, 60 years old, developed shaking chills and protracted fever as a probable complication of thrombophlebitis following a recent saphenous vein ligation for varicose veins and ulcer. Blood cultures repeatedly yielded a heavy growth (about 500 colonies per cc.) of *Staphylococcus aureus* (coagulase +).

On admission on February 7, 1944, he was gravely ill and in a stupor. Many petechiae were present on the conjunctivae and extremities. A harsh systolic aortic murmur, audible in the neck and arms, was present without thrill. After the first week the auscultatory and physical signs of aortic insufficiency developed. Penicillin was given intramuscularly at intervals of 2 to 4 hours, in doses of 20,000 units for the first 3 days, and in 10,000 unit doses for 18 days longer; the total given was 1,940,000 units. Fever and petechiae disappeared at the end of the first week and did not return. All of 8 blood cultures after the inception of treatment were sterile. However, toward the end of the second week of treatment attacks of acute paroxysmal dyspnea and pulmonary edema appeared, and progressive and intractable congestive failure progressed in spite of the usual therapeutic efforts to control heart failure with digitalis, oxygen, salt restriction, and diuretic drugs. The patient died on the 21st hospital day in acute pulmonary edema.

Autopsy was performed 5 hours after death by Dr. A. Newman. The significant findings follow.

Gross Examination. The heart weighed 515 gm. and all chambers

were dilated. The right ventricle measured 5 mm. in thickness; the left, 12 mm. The circumferences of the tricuspid, pulmonic, mitral, and aortic valve rings were 13, 7, 9, and 7 cm., respectively. Irregular fibrous and calcified elevations were present on the tricuspid and mitral leaflets along the line of closure. The chordae tendineae of the mitral valve were thickened and shortened and a few were fused and inserted into the thickened free edge of the leaflets. The aortic valve cusps were thickened, calcified, and fused; the right posterior cusp was swollen and red, and the aortic surface of the cusp was covered by a red-brown, friable thrombus. A small aneurysm was present in the posterior wall of the aorta above the valve ring. The mouth of the aneurysm was 4 mm. in diameter and the structure measured 6 mm. in diameter. A zone of exudate was seen about the aneurysm in the soft tissue at the base of the heart.

The lungs weighed 630 and 690 gm., and yielded foamy fluid when cut; there were no infarcts. The spleen was soft and friable and weighed 370 gm. The right and left kidneys weighed 190 and 218 gm., respectively. The cut section revealed a swollen cortex and indistinct tubular markings. The liver, which weighed 1475 gm., was brown and the lobular markings were accentuated.

Microscopic Examination. On microscopic examination the myocardium was well preserved and the cells hypertrophied. The leaflets of the mitral valve were thickened by hyalinized connective tissue and were devoid of cellular exudate. Section of the right posterior cusp of the aortic valve showed masses of bright eosinophilic material within the cusp undergoing organization and calcification (Fig. 1). Fresh and organizing thrombi were present on the endocardial surface over these lesions. No organisms were detectable with special bacterial stains. The aneurysm in the aorta was lined by thrombus and the wall was composed of granulation tissue containing numerous polymorphonuclear leukocytes. This exudate extended into the peri-aortic tissues. No bacteria could be demonstrated in the wall of the aneurysm, in the surrounding tissues or in the thrombus in the lumen by the Brown-Brenn Gram stain.[1] Small zones of fibrinopurulent pneumonia were present in the tissues about the bronchi. The lungs, liver, and spleen showed acute and chronic passive congestion. Rare adhesions between the glomerular tuft and Bowman's capsule were found in the kidney, but no other lesions were present to indicate focal embolic nephritis.

Bacteriologic Examination. Post-mortem blood cultures were sterile, as were cultures of the aortic valve. Splenic culture yielded *Bacillus coli.*

Anatomic Diagnoses. Organizing vegetation of aortic valve; mycotic

aneurysm of ascending aorta; cardiac dilatation and hypertrophy; chronic rheumatic mitral valvulitis; acute and chronic passive congestion of viscera; hydrothorax (bilateral); focal pneumonia. (*Staphylococcus aureus* septicemia present 3 weeks before death; diabetes mellitus.)

Death in this case resulted from intractable congestive cardiac failure.

· *Case 2*

T. J., a woman, 22 years old, had had chorea 11 years before her fatal illness, followed by acute nephritis 1 year later. Three days before admission she became acutely ill with fever (104° F.), epistaxis, sweats, and headache. During the next 2 days pain developed in the left foot and wrist, and an Osler's node appeared on the left thumb.

She was acutely ill with high fever, tachycardia, and a blood pressure of 80/50 mm. Hg. About 12 petechiae were found in the skin, under one eyelid, and under one fingernail. The left wrist was swollen and tender. The moderately enlarged heart presented a fairly loud and slightly coarse systolic murmur at the apex. Laboratory examinations disclosed a moderate anemia, a relative polymorphonuclear leukocytosis, microscopic hematuria, and a few granular casts in the urinary sediment. Blood culture was heavily positive for hemolytic *Staph. aureus* (coagulase +, mannite +). The admission diagnosis was rheumatic heart disease with mitral valvulitis and insufficiency, and acute bacterial endocarditis probably on the mitral valve.

Treatment consisted of immediate transfusion and institution of massive penicillin therapy by continuous intravenous drip. The dosage of penicillin began at 240,000 units per day but was soon increased to 500,000 and then to 1,000,000 units per day when it was found that the infecting organism was relatively resistant to penicillin (0.05 units per cc. were required to inhibit growth *in vitro*), and that smaller daily doses failed to yield serum penicillin concentrations above 0.16 units per cc. of serum (due, probably, to a high penicillin "K" content of the product). Treatment was discontinued after 31 days during which 18,520,000 units of penicillin had been administered.

Although the patient improved symptomatically, and bacteremia and embolic phenomena disappeared early·in the course of treatment and remained absent for· more than 1 month thereafter, fever of moderate grade persisted. During the last 3 weeks of life she again became seriously ill with symptoms and signs of pleuritis. and pneumonitis (no pathogenic bacteria could be recovered from the sputum). These manifestations were progressive. Dyspnea, cyanosis, and edema developed in spite of the administration of digitalis, and the patient died on the 70th hospital day. Seventeen successive blood cultures in the course of 5 weeks following the termination of penicillin treatment had been sterile, but the last blood culture, 3 days before death, yielded a scant growth of hemolytic *Staph. aureus*.

Autopsy performed 11 hours after death by Dr. B. Lown revealed the following significant findings:

Gross Examination. The heart weighed 310 gm., and only the left auricle was dilated. The right ventricle measured 4 mm.; the left, 12 mm. in thickness. The tricuspid and pulmonic valves measured 11.5 cm. and 6.5 cm., respectively, and showed no changes. The chordae tendineae of the mitral valve were thickened, pale, and opaque,

and the fused cords inserted upon the rounded and thickened free edges of the leaflets. A small, flat vegetation, measuring 4 by 7 mm., was found on the anterior leaflet of the valve. On the posterior leaflet was a hard, white, raised, glistening mass measuring 1.5 by 2 by 1 cm. This vegetation occluded a large portion of the valve orifice, which measured 6.7 cm. in circumference (Fig. 2). The aortic valve was 5.5 cm. in circumference and was composed of delicate cusps with no fusion of the commissures.

The lungs each weighed 700 gm. Both upper and lower lobes were dark red and were firm and dry when cut. The right middle lobe and the bronchi exuded frothy fluid. The vessels were free of occlusions. The spleen weighed 175 gm. and was not remarkable except for a large infarct in the central portion. The liver weighed 1435 gm. and the lobular architecture was accentuated. The right kidney weighed 180 gm. and was congested; the left, 75 gm. A large, irregular, opaque, gray-white infarct occupied a major portion of this kidney.

Microscopic Examination. On microscopic examination the myocardial cells were well preserved and not hypertrophic. Throughout the interstitial tissue were numerous Aschoff bodies composed of large mononuclear cells with characteristic owl-eyed nuclei, multinucleated giant cells, and eosinophilic collagen. The vegetations on the mitral valve consisted of endothelium-covered foci of eosinophilic material, surrounded by granulation tissue and hyalinized fibrous connective tissue (Fig. 3). No organisms were seen in bacterial stains of these lesions.

The alveolar walls in the diffuse areas of pneumonia were thickened by edema fluid and polymorphonuclear and mononuclear leukocytes. An eosinophilic membrane lined the alveoli and respiratory bronchioles. Within the alveoli were mononuclear cells containing blood pigment, occasional polymorphonuclear leukocytes, and numerous red blood cells. Occasional hyaline thrombi were noted in capillaries and arterioles. Organizing infarcts were present in the spleen and left kidney.

Bacteriologic Examination. Cultures of the vegetation on the mitral valve yielded no growth. Culture of the right lower pulmonary lobe yielded hemolytic *Staph. aureus* (coagulase +, mannite +).

Anatomic Diagnoses. Active rheumatic myocarditis; healed rheumatic mitral valvulitis; healing vegetative endocarditis of mitral valve; infarcts of spleen and left kidney; congestion of viscera; hemorrhagic and interstitial pneumonia with capillary thrombi (compatible with rheumatic pneumonia).

Death evidently was due to active rheumatic myocarditis, pneumonia, and congestive failure of the heart.

Case 3

J. F., a female, 64 years old, had been ill for 4 days with fever and stupor that failed to respond to sulfonamide drugs, whereupon she was admitted to the hospital on January 18, 1944. Except for asymptomatic hypertension of at least 3 years' duration, she denied illness in the past. The patient was comatose, had fever of 103° F., and tachycardia of 140 per minute. There were signs of meningeal irritation, basal pulmonary edema, purulent conjunctivitis, and a small draining pustule at the base of the left great toe. Petechiae were noted on the eyelids. The spinal fluid was sterile but contained 24 polymorphonuclear leukocytes per cmm., and the protein content was 97.6 mg. per cent. Two successive blood cultures yielded *Staph. aureus* (coagulase +). The same organism was grown from the conjunctival exudate, the pustule on the toe, and from urine which also contained considerable albumin and pus.

Penicillin was begun on the third day in doses of 10,000 units every 2 hours intramuscularly for 3 days, when the doses were doubled for 6 days, and then reduced again to 10,000 units every 2 hours for 5 days longer. A total of 1,390,000 units was given in 15 days. Although petechiae disappeared and the blood remained sterile after the second day of treatment, the patient continued moderately febrile and acutely ill with renal failure characterized by oliguria, azotemia, and the electrolyte pattern of acidosis. Signs of congestive failure were controlled with digitalis. Although the spinal fluid cleared, convulsions developed and the patient unexpectedly was found dead 5 days after penicillin treatment had been terminated.

Autopsy performed 4 hours after death by Dr. H. Arnold revealed the following findings:

Gross Examination. The heart weighed 380 gm. The left auricle was dilated and lined by thick, white, wrinkled endocardium. The mitral orifice was reduced to a crescentic slit, 1.5 cm. in length, surrounded by cartilaginous opaque leaflets 3 mm. in thickness. On the auricular surface of the anterior leaflet, 2 mm. from the line of closure, was a friable and soft gray-brown vegetation 0.75 cm. in diameter. A small hemorrhage was present in the anterior leaflet near the line of closure. The chordae tendineae were thick, short, and fused, and they inserted into the rounded free edge of the valve. The tips of the papillary muscles were scarred. The endocardium of the enlarged and hypertrophied left ventricle was transparent. The aortic valve cusps were stiff and inelastic but were not fused. No gross changes were present in the right chambers of the heart.

The spleen weighed 140 gm. and was soft and friable. The kidneys each weighed 240 gm. The decapsulated surfaces were finely granular and mottled yellow-brown. Numerous small abscesses were scattered throughout cortex and medulla. The parenchyma was swollen and had a parboiled appearance. The left lobe of the liver contained several encapsulated abscesses less than 1 cm. in diameter. The brain contained an abscess measuring 1.5 by 1 cm. in the left frontal lobe and a smaller abscess in the left occipital lobe.

Microscopic Examination. Brown-Brenn Gram stains [1] of the or-

ganizing mass of fibrin, red blood cells, and polymorphonuclear leukocytes on the auricular surface of the fibrotic mitral leaflet revealed no organisms (Figs. 4 and 5). Gram-positive cocci were demonstrated in abscesses of liver, kidneys, and brain. In addition, lesions of focal embolic nephritis were present in the kidneys.

Bacteriologic Examination. Culture of the blood at necropsy yielded no growth. *Staph. aureus* was grown from the liver abscess.

Anatomic Diagnoses. Healed rheumatic endocarditis with mitral stenosis; healing vegetative mitral endocarditis (vegetations free of organisms); abscesses in liver, kidneys, and brain; focal embolic nephritis; edema; decubital ulcers.

Death in this case seemed attributable to cerebral and renal abscesses and renal failure.

Case 4 *

R. B. was a female, 18 years old, with known mitral stenosis. In May, 1944, she developed subacute bacterial endocarditis due to *Streptococcus viridans.* She was treated with 12,000,000 units of penicillin given in 54 days, largely by continuous intravenous drip. From December 9, 1944, to June 14, 1945, she was ambulatory, entirely asymptomatic and afebrile, and all of 10 blood cultures in this interval were sterile. Two weeks following the extraction of an abscessed tooth on June 14, 1945, slight fever and malaise reappeared and cultures of the blood were again heavily positive for *Str. viridans.* Immediate treatment with penicillin by continuous intravenous infusion of 240,000 units daily for 22 days again brought about apparent clinical and bacteriologic cure. Late in August, symptoms and signs of cerebral and meningeal infection suddenly developed, and cultures of the blood were persistently positive for *Candida albicans.* Therapy with penicillin in massive doses for 3 weeks, and later sulfadiazine and potassium iodide, proved ineffectual. Death occurred on October 5, 1945.

An autopsy was performed 3 hours after death by Dr. H. D. Axilrod.

Summary of Anatomic Diagnoses. Healed mitral and aortic endocarditis; mitral stenosis; myocardial fibrosis; cardiac hypertrophy and dilatation; chronic passive congestion of viscera; healed vegetative endocarditis, Figure 6 (residuum of previously cured *Streptococcus viridans* endocarditis); healed infarcts of spleen and kidneys; acute vegetative endocarditis involving chordae tendineae of mitral valve and left auricular endocardium (*Candida albicans*); rupture of chordae tendineae; acute and organizing focal myocardial necrosis; fresh infarcts in spleen, kidneys, and right basal ganglia; focal embolic glomerulonephritis; petechiae in left conjunctiva; multiple granulomata of brain; ruptured mycotic aneurysm of left middle cerebral artery; subarachnoid hemorrhage; granulomatous leptomeningitis (*Candida albi-*

---

* This case has been reported in detail elsewhere (Geiger, A. J., Wenner, H. A., Axilrod, H. D., and Durlacher, S. H. Mycotic endocarditis and meningitis; report of case due to *Monilia albicans. Yale J. Biol. & Med.*, 1946, 18, 259–268.

cans); acute tracheitis and bronchitis; acute and organizing focal pneumonia, bilateral.

Bacteriologic Examination. Candida albicans was ·recovered from the lungs, spleen, kidneys, brain, liver, blood, and the vegetation on the mitral valve.

Death obviously resulted from overwhelming systemic dissemination of infection with *Candida albicans*.

COMMENT

The four cases herein described constitute anatomic proof that the infective component of bacterial endocarditis is eradicable with penicillin. Such pathologic observations, together with the favorable clinical experiences of the past few years, complete the chain of evidence that bacterial endocarditis is curable.

The nature of the healing process in its several stages was well seen in histologic study of the vegetations. A mass of granulation tissue and hyalinized connective tissue, surrounding eosinophilic material and covered by endothelium (case 2, Fig. 3), apparently represented an early phase. Also obviously early was the organizing mass of fibrin, erythrocytes, and leukocytes that composed the vegetation in another instance (case 3, Figs. 4 and 5). More advanced organization and the deposition of calcium salts within the vegetation appeared to indicate a later stage (case 1, Fig. 1). Finally, the completely healed lesion was exhibited by a pale, hard, and smoothly endothelialized mass of dense connective tissue and areas of calcification (case 4, Fig. 6). It is noteworthy that bacteria were neither seen in, nor cultivated from, the depths of any of these lesions, whether healing or healed, proving that vegetations actually become free of bacteria. Furthermore, the character of the healing or healed lesion in these cases resembles that described by Rosenblatt and Loewe [2] in two cases that had been treated with penicillin and heparin. The absence of thrombotic accretions on the vegetations implies that anticoagulant medication is not an essential adjunct of therapy.

Focal embolic nephritis was not evident in cases 1 and 2, but was present in cases 3 and 4; the last was an instance of active mycotic infection with endocarditis and systemic dissemination at death.

The fatal outcome in case 1 resulted from congestive failure of the heart which developed abruptly and progressed intractably and rapidly in spite of all therapeutic efforts. This result was probably related to the aortic valvular location of the destructive bacterial endocarditic process, with the consequent sudden imposition of undue strain upon a left ventricular muscle that had insufficient opportunity to compensate

through hypertrophy. It is interesting that the two cases of healed bacterial endocarditis studied post-mortem and reported by Rosenblatt and Loewe [2] also showed serious destruction of the aortic valves and both patients had died of congestive failure that progressed relentlessly. There are two obvious implications in this observation: (1) In cases of bacterial endocarditis involving the aortic valve, it is essential that effective antibacterial treatment be instituted at the earliest opportunity, particularly in the presence of acute endocardial infection with rapidly destructive organisms such as the staphylococcus; (2) Convalescence in patients with vegetations on the aortic valve should be prolonged, and return to physical activity should be undertaken slowly, under careful observation, and with skillful institution of the usual measures employed in the control of congestive failure.

SUMMARY

Four cases of bacterial endocarditis, treated with penicillin, presented both histologic and bacteriologic evidence of cure. The healing process evidently results in the disappearance of bacteria from the lesions, not merely their incarceration within the vegetations.

REFERENCES

1. Brown, J. H., and Brenn, L. A method for the differential staining of gram-positive and gram-negative bacteria in tissue sections. *Bull. Johns Hopkins Hosp.*, 1931, 48, 69–73.

2. Rosenblatt, P., and Loewe, L. Healed subacute bacterial endocarditis. *Arch. Int. Med.*, 1945, 76, 1–10.

DESCRIPTION OF PLATES

PLATE 148

FIG. 1. Case 1. Tissue of organization invading compact fibrin mass in center of vegetation. Hematoxylin and eosin stain. × 175.

FIG. 2. Case 2. Mitral valve with flat vegetation on anterior leaflet and large round mass on auricular surface of posterior leaflet.
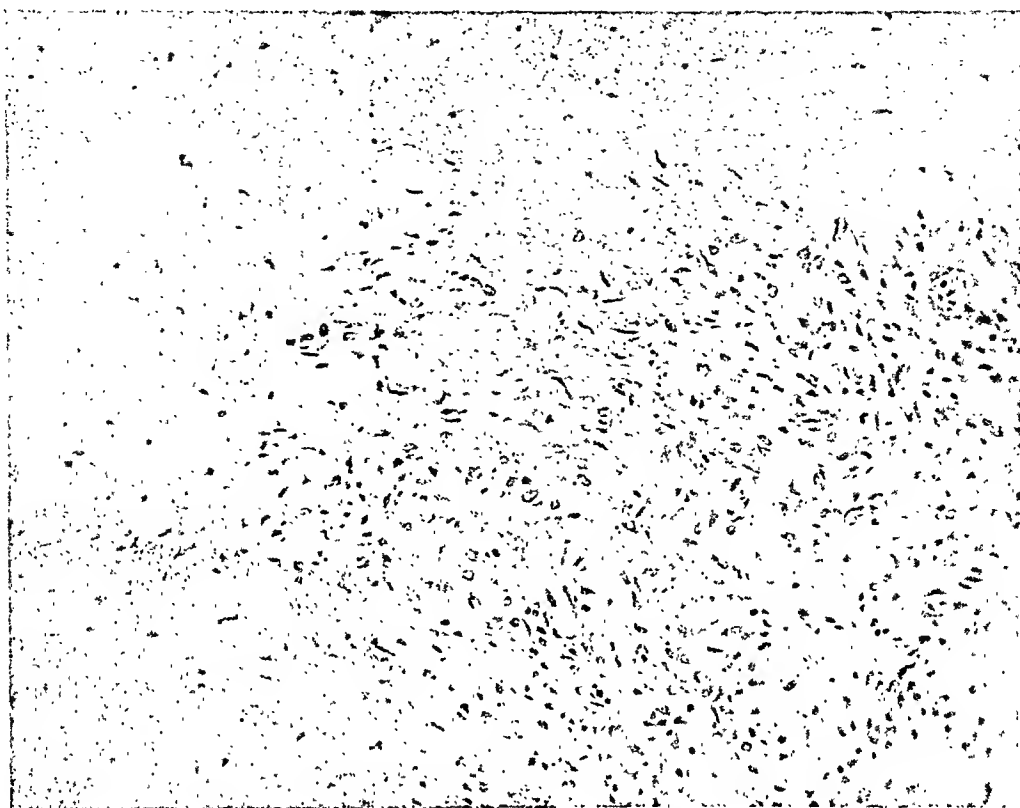
Geiger and Durlacher   Endocardial Vegetations Following Penicillin

PLATE 149

FIG. 3. Case 2. Process of organization extending into fibrin mass in center of vegetation on posterior leaflet. Hematoxylin and eosin stain. $\times$ 140.

FIG. 4. Case 3. Central portion of vegetation consisting of fibrin, platelets, red blood cells, and leukocytes. Hematoxylin and eosin stain. $\times$ 225.

3

4

Geiger and Durlacher   Endocardial Vegetations Following Penicillin
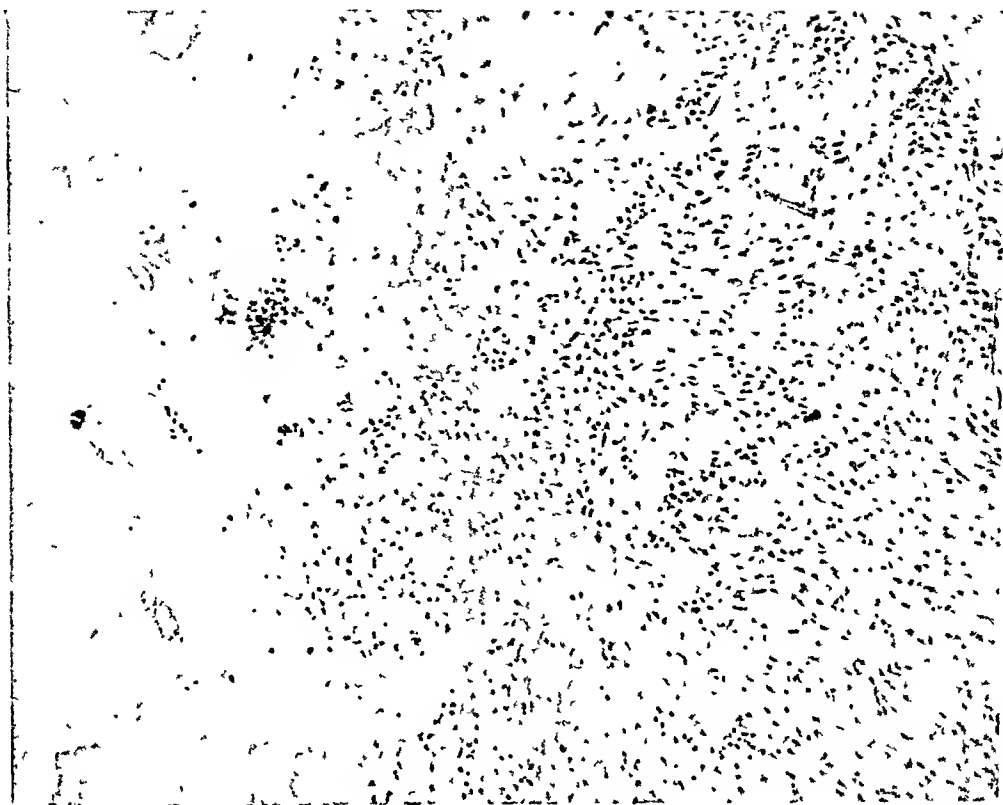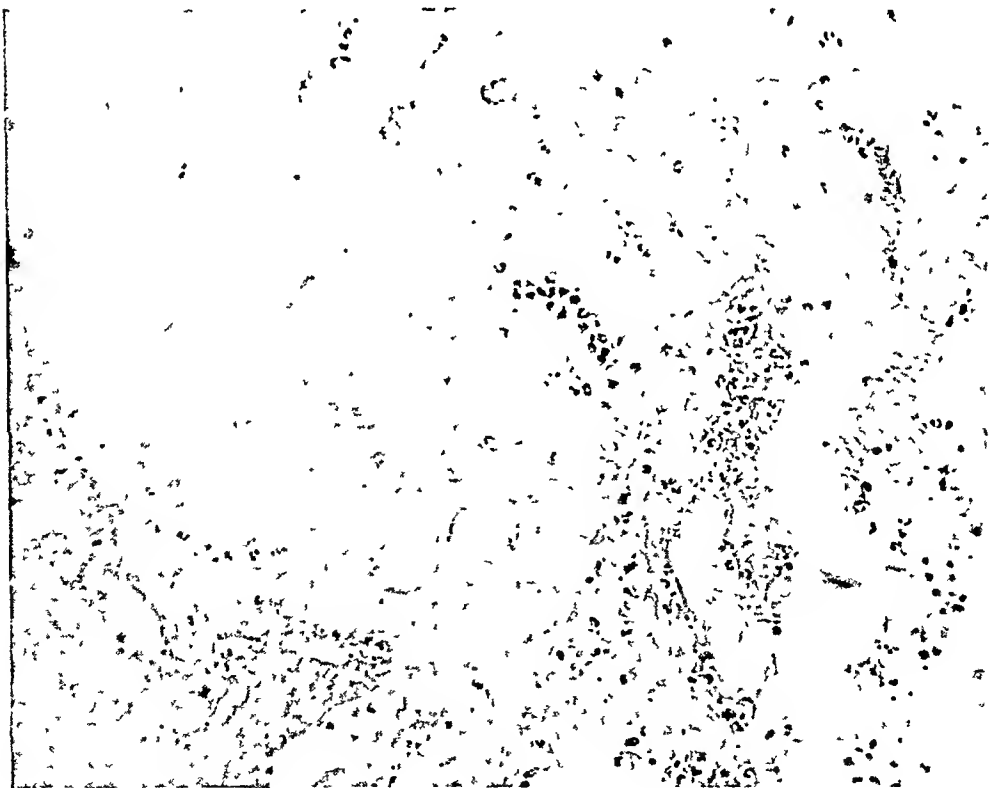
PLATE 150

FIG. 5. Case 3. Tissue resulting from organization and covered by endothelium on auricular surface of vegetation. There is no evidence of recent thrombosis. Hematoxylin and eosin stain. $\times$ 235.

FIG. 6. Case 4. Dense fibrous connective tissue and calcified thrombus on endothelial surface of left auricle above line of attachment of posterior leaflet of mitral valve. Hematoxylin and eosin stain. $\times$ 130.

Geiger and Durlacher

Endocardial Vegetations Following Penicillin
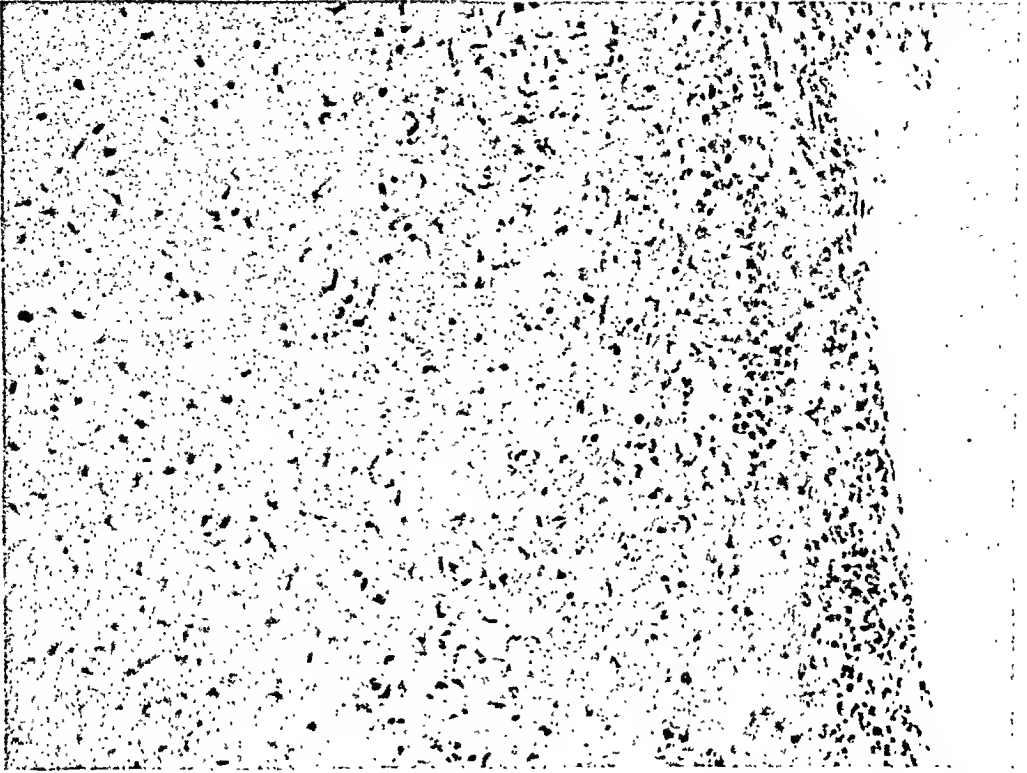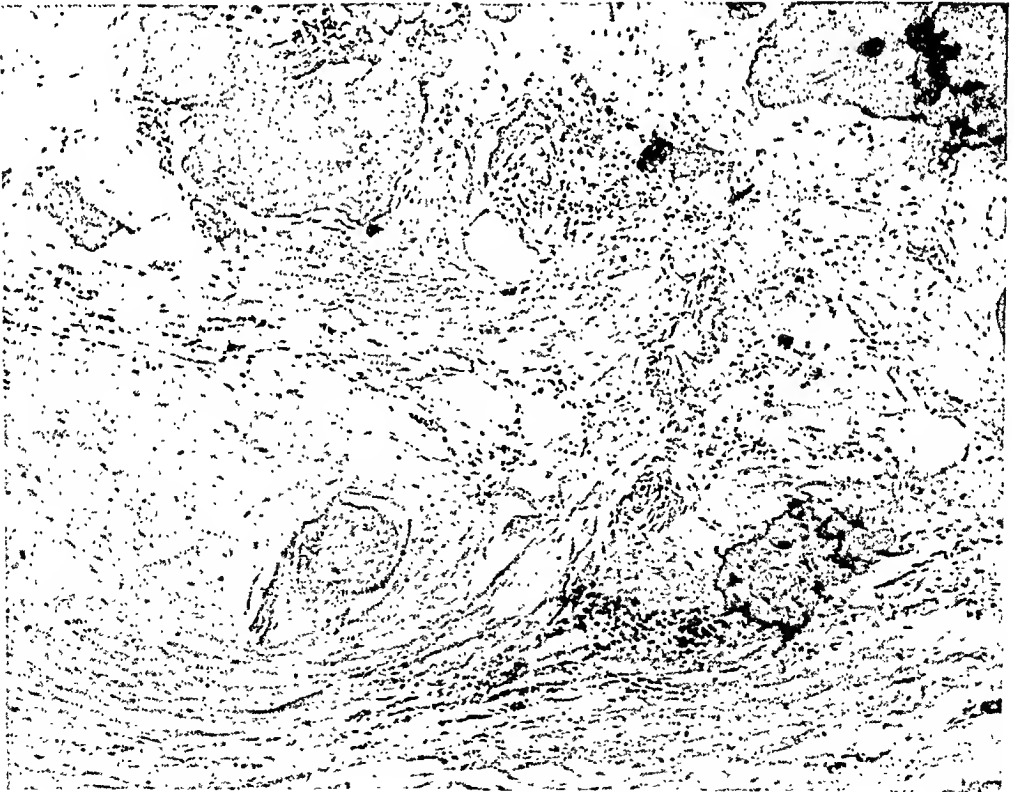
STUDIES ON EXPERIMENTAL PHOSGENE POISONING

I. The Pathologic Anatomy of Phosgene Poisoning, with Special Reference to the Early and Late Phases *

Dale R. Coman, M.D., H. D. Bruner, M.D., Robert C. Horn, Jr., M.D., Melvin Friedman, M.D., Robert D. Boche, Ph.D., Miles D. McCarthy, Ph.D., Mary H. Gibbon, and Julius Schultz, Ph.D.

(From the Harrison Department of Surgical Research and the Department of Pathology, School of Medicine, University of Pennsylvania, Philadelphia 14, Pa.)

Except for sporadic observations, study of the development of the lesions constituting "typical" phosgene poisoning has been neglected, and one of the chief purposes of this report is to close this hiatus. Obviously, a systematic analysis of the sequence of tissue changes from the end of gassing onward is prerequisite to the comprehension of the symptomatology and the abnormal physiology of phosgene poisoning. Another object is to describe both the regressive-reparative and the degenerative processes found in animals surviving the critical 72 hour post-exposure period, which has received more, but still inadequate, attention in the past. The massively edematous lung of acute phosgene poisoning will be treated briefly inasmuch as the present studies add little to what is already in the literature.

It is only in connection with the acute or critical phase of the damage (6 to 72 hours following gassing) that a comparison can be drawn between the findings in men poisoned in industrial accidents or in war and the lesions observed in animals poisoned under controlled conditions. As will be pointed out, there is imperfect agreement between the two, and reasons for this may be traced to one or more of the following: (a) The prevalence of respiratory infections during 1915 to 1920 may have complicated or altered the response of the lung to injury, particularly in men surviving longer than 36 hours. (b) In both accidental and field exposures, it has never been possible to estimate with any reliability the dose to which the victim was exposed, and it is known that exposure to extremely high or low concentrations for a given time will result in anomalous lesions.[1-3] (c) Since phosgene was rarely used in World War I,[4-6] except in combination with some other gas, descriptions of the lesions found in casualties may not be applicable to pure phosgene injury. (d) The conditions existing during the interval between death and autopsy were not specified in the reports on human material; this stands in direct contrast to experimental work. (e) Species differences may, in part, account for variation in response

to injury. In this connection it may be significant to the interpretation of the cardiovascular abnormalities that the animal is more resistant to anoxia than is man.[7-9] On the whole, however, agreement among the various pathologic data is more satisfactory than that between physiologic experiments and inferences from clinical observation.

The data in this report, it is believed, make it clear that the reaction of the lung to phosgene is a type reaction common to several other injurious agents, rather than a specific response.

MATERIALS AND METHODS

Exposure of the animals to phosgene was carried out in an 850 liter chamber through which specified phosgene-air mixtures were drawn at rates of 300 to 600 liters per minute, depending on the size and number of animals. The concentration of phosgene in the mixture was sampled by absorbing the phosgene from a measured fraction in an alcoholic solution of alkali, subsequently analyzed for total chloride.

Two technics of exposure were used in these studies; they appear to give very similar, if not identical, clinical and pathologic findings:

(1) The desired concentration of phosgene was built up in the chamber, whereupon the caged animals were inserted into the gas mixture via a double door slide arrangement; after a time predetermined to result in a desired mortality at a given phosgene concentration, the animals were withdrawn into a cleansing air current. The phosgene concentration was sampled synchronously with the exposure. This "push-pull" method simulates field exposures and saves time, but causes the operators to work hurriedly in dangerously high concentrations of phosgene; it is not suited for species such as the rabbit and goat which are able to suspend breathing for a considerable time.

(2) The "rise-fall" method was used mainly because of greater convenience and safety. The caged animals were placed in the chamber and then the admixture of phosgene with inflowing air was begun. This results in an exponential rise of concentration which was calculated and found to reach equilibrium in 10 minutes. After a given time at this equilibrium, the phosgene was turned off, whereupon the phosgene concentration in the chamber fell by the same exponent, but reciprocally. The duration of exposure was taken to be the sum of the duration of constant composition plus 10 minutes. The phosgene concentration was sampled throughout the period of constant concentration.

The phosgene used in these experiments was stated to be better than 99.9 per cent pure $COCl_2$, the remainder being HCl.

The dose of phosgene is expressed conventionally as the product of mean concentration in mg. per liter of air (C) and duration of exposure in minutes (T). This convention is based on the assumption that minute breathing volume, and hence the intake of gas, is proportional to the metabolism which in turn is a function of the weight; it is comparable to the usual dose system based on body weight.[10] Therefore, the adults of a species, regardless of size, are assumed to receive the same proportional dose of a given CT product. To anticipate the results, analyses of the mortality data support this convention; in any event "individual susceptibility" and behavior during exposure are regarded as more important variables.

The dogs, cats, rabbits, and guinea-pigs employed were adults of mongrel origin, whereas the rats, weighing 150 to 225 gm., were of the genetically homogeneous Wistar strain, obtained from the Institute as needed. The latter were secured in an effort to reduce "individual susceptibility" to a minimum. The other animals were not used until an isolation period had shown them to be free of infection and eating well. All of the small animals of an experiment were gassed simultaneously in individual cages, while the dogs were gassed in pairs or in fours in cages.

The dogs were sacrificed by rapid arteriotomy under amytal or local anesthesia and the small animals by thoracotomy under nembutal anesthesia. A number of ungassed animals of each species were similarly sacrificed for comparison.

The animals were autopsied at once after death (with the exception of a few kept overnight at −4° C.) and representative tissues taken for histologic examination. The lungs were removed *in toto* after ligation at the hilum, and multiple sections, or the whole lung in the case of the rat and guinea-pig, were fixed at once in Helley's fluid. The tissues embedded in paraffin were sectioned at 7.5 μ and stained with hematoxylin and eosin or special stains as indicated.

The animals listed in the tables which follow are representative of the larger number which were gassed for histologic study. Further, the data agree with those from more than 1,050 dogs and 2,500 rats used in this laboratory in experiments on phosgene poisoning.

RESULTS

To facilitate description, the course of phosgene poisoning has been divided into three phases, which are somewhat different from those of Winternitz.[11] It must be emphasized that such a division is arbitrary, since the sequence of events initiated by exposure to phosgene is con-

TABLE I

Evaluation of Acute Pathologic Changes in the Lungs of Dogs Exposed to a Moderate Concentration of Phosgene (L.D.₉₀₊) by the "Rise-Fall" Technic

Dog no.	Conc. of CG* (mg./L.)	Time after "CG on" (hours)	Sacrificed (S) or died (D)	Emphysema	Congestion of lung	Alveolar edema	Mucosa of bronchioles; swelling, sloughing, and/or plugging	Bronchiolar constriction	Lung/body weight (× 100)
118	0.49	0:38	S	++	o, o	o	±,+		1.13
117	0.49	1:38	S	++	o, o	±,+	+,++		1.25
529	0.64	1:39	S	++,+	o, o	++,++	+,++	++,+	1.20
539	0.50	1:52	S	+,+	o,+	++,+	++,++	++,±	1.60
560	0.52	2:39	S	+++,+++	+,++	++,++	+++,+	+,±	2.11
728	0.64	2:46	S	+++,+++	o, o	+,++	+++,+	Dl‡,o	1.36
439	0.82	3:36	S	+++,+++	+,+	+++,+++	+,++	o	1.45
551	0.58	3:46	S	+++,+	+++,++	+++,+	+++,+	±,+	1.32
642	0.48	3:52	S	+++,+++	+,++	++,+	+,++	o	1.82
566	0.58	3:52	S	++,+	++,+	++,+	+++,+	Dl, o	1.46
579	0.67	4:33	S	+++,+++	++,+	++,++	+,+†	Dl, o	2.24
440	0.45	5:07	S	+++,+++	+,++	+++,+	+,++	Dl, o	1.89
530	0.64	5:31	S	++,+++	+,++	++,+	+,++	Dl, o	2.80
043	0.37	6:18	D	+++,+++	+,+	+,+	+,+	o	3.47
413	0.76	7:32	S	+++,+++	+,++	+++,+++	+++,+	Dl, o	2.69
447	0.76	8:17	D	+++,+++	+,++	+++,++	+++,+	Dl, o	2.10
550	0.60	8:35	S	+++,+++	+,+	+,+	+++,+†	Dl, o	3.52

* CG = phosgene. Total theoretical time of exposure = 30 min.
† Beginning "bronchopneumonia" suggested by leukocytic invasion.
‡ Dilated.

TABLE II

Evaluation* of Acute Pathologic Changes in the Lungs of Dogs Exposed to a High Concentration of Phosgene (L.D.$_{50}$ approx.) by the "Push-Pull" Technic

Dog no.	Conc. of CG† (mg./L.)	Time after "CG on" (hours)	Sacrificed (S) or died (D)	Emphysema	Congestion of lung	Alveolar edema	Mucosa of bronchioles; swelling, sloughing, and/or plugging	Bronchiolar constriction	Lung/body weight (×100)
815	2.98	0:08	S	++	±	o	o	±	1.04
795	3.30	0:08	S	+++	o	o	o	±	1.30
807	3.33	0:20	S	+++	±	o	o	±, +	1.19
819	2.95	0:31	S	+++	o	o	+	±	0.92
817	2.98	0:33	S	++	o	o	o	±, +	1.12
818	2.95	1:01	S	+++	++	o	++	±	0.97
816	2.98	1:04	S	+++	+++	o	o	+	1.06
808	3.33	1:42	S	++++	+++	o	++	±	1.18
796	3.30	2:04	S	++++	o	o	++	±	1.51
820	2.95	4:09	S	++++	+++	o	+	o	1.82
809	3.33	4:10	D	++++	+++	+	++	Dl§	2.50
849	3.20	4:20	S	++++	+++	++	+++	Dl	4.65
797	3.30	5:19	D	+++	+++	++	+++	Dl	2.50
390	3.55	6:14	D	+++	+++	++	+++	Dl	5.91
287	3.31	6:20	D	++	+++	++	+++	Dl	2.92
243	3.22	7:00	D	++	+++	+++	+++	Dl	4.26
279	3.22	7:05	D	++	+++	+++	+++	Dl	4.48
254	3.25	8:54	D	+	+++	+++	+++	Dl	2.82

* By R. H. only.

† Time of exposure = 3 min.

‡ Beginning "bronchopneumonia" suggested by leukocytic invasion.

§ Dilated.

tinuous and ends either in death or in repair and survival. These three artificial divisions are, chronologically:

1. The incipient phase which extends from gassing up to 2 to 6 hours.

2. The critical phase in which the majority of deaths occur; in survivors this phase ends about 3 days after exposure.

3. The regressive and reparative phase which extends from the fourth day onward; the time of completion of repair is uncertain. The residua of phosgene poisoning are regarded as the terminal stage of repair.

The development of the typical lesions from controlled exposure to pure phosgene has been condensed in Tables I through IV. In the tables o, $\pm$, $+$, $++$, and $+++$ are to be read as none, doubtful, mild, moderate, and severe, respectively. Each designation represents the independent evaluation of the sections by two pathologists (D. R. C. and R. C. H., Jr.), and where they disagreed, both evaluations were entered. Agreement is remarkably close considering that each entry is the summed evaluation of all sections of the lung of an animal. "CG" is the convention for phosgene.

The method involves a sampling procedure and is susceptible to all of the errors inherent in sampling a population, since a given section may constitute but a small portion of a lung. This is important since it has been observed that the periphery of the lung may not show the same degree of radio-opacity as the hilar region.[12]

The Incipient Phase of Phosgene Poisoning

The pulmonary changes in the incipient phase are those which precede clinically-evident pulmonary edema. They are emphysema, necrosis and sloughing of bronchiolar mucosa, perivascular and peribronchial edema, congestion, thickening of the alveolar membranes, and perhaps bronchial-bronchiolar constriction.

Emphysema. As indicated in the tables, emphysema was the earliest prominent lesion in animals exposed to potentially lethal doses of phosgene. Tables I and II dealing with dogs gassed by different technics indicate that this very early, if not immediate, change is not a technical artifact; the data on the other species confirm this. Not only is it probably the earliest anatomic change, but it appears to attain full development prior to the other changes.

Grossly, emphysema appeared as small pale patches on the surfaces of otherwise unaltered lungs in the earliest specimens. Microscopically (Fig. 1), the distribution of emphysema was patchy and lobular. The alveolar septa were extremely thin and at times fragmented, while the

capillaries were thin and relatively bloodless. Small areas of advanced patchy atelectasis, as seen later, were not apparent in this phase.

Bronchiolar Constriction. The criteria adopted for recognition of constriction of the airways were thickening of the musculature and folding and compression of the mucosa, so as to cause obvious narrowing of the lumen (Fig. 2). On this basis constriction was relatively infrequent (Tables I through IV), but when found it was usually restricted to the finer air passages, in particular, the terminal bronchioles. Of the several species, only the guinea-pig showed conspicuous narrowing. In nearly all instances, the constriction was found only in animals sacrificed within 2 hours after gassing; later the bronchioles were dilated rather than constricted (Fig. 3). Thus, in general, bronchiolar constriction, to whatever degree it did occur, was transient and soon supplanted by dilatation.

Necrosis and Sloughing of the Bronchiolar Mucosa. Swelling of the mucosa of the small and medium-sized bronchioles with sloughing of the epithelium to form plugs in the lumina was found in the earliest stages. It was constantly present, although overshadowed by emphysema. The epithelium sloughed in shreds and irregular patches, and some areas which had not sloughed showed signs of incipient necrosis. The slough together with mucus formed more or less solid masses which were mobile, since a mass might be found to plug the lumen of an intact bronchiole (Fig. 4) or an air passage might be free of all mucous membrane. Such lesions were usually confined to bronchioles proximal to the respiratory bronchioles.

The number of plugged air passages varied from section to section and from animal to animal. Correlation of the location of such plugs with emphysematous lobules was not feasible without serial sections and this was not attempted. The sloughs were believed not to be artifacts.

Perivascular and Peribronchial Edema. Lace-like expansions of the connective tissue around the medium and larger blood vessels and air tubes was another constant early change (Fig. 5). These cuffs were formed by distention of the interstices of the connective tissue with a lightly staining eosinophilic material. These lacunoid spaces were distinct from the lymphatics, which were not particularly distended in this phase. The site of origin of this fluid was not detected, but it clearly was not alveolar because the cuffs faded out as the vessels became finer and were found prior to alveolar edema. Causal relations of this extra-alveolar edema with other anatomic changes could not be established.

Congestion and Alveolar Edema. Dilatation and engorgement of

TABLE III

Evaluation of the Acute Pathologic Changes in the Lungs of Rats after Exposure to L.D.$_{90-100}$ C×T's of Phosgene by the "Push-Pull" Technic

C-T data	Time after "CG on" (hours)	Sacrificed (S) or died (D)	Emphysema	Congestion of lung	Alveolar edema	Mucosa of bronchioles; swelling, sloughing, and/or plugging	Bronchiolar constriction	Lung/body weight (× 100)
Rat group 38 C=0.30 mg./L. T=13.5 min.	0:16	S	++++	+, +++	o	+, ++	o,	1.04
	2:13	S	++++	±, ++, ++	++++	++, +++	±±,	1.10
	4:13	D	++++	++, ++	±, ++	+++, +++	o,	3.46
	5:13	D	++++	++, +	++	++	o	3.28
	9:13	D	++	+	+	+++		3.27
Rat group 51 C=0.31 mg./L. T=10.5 min.	0:15	S	++	o	o	+, ++	o,	0.85
	0:25	S	+++++	+++	o	+++, ++++	o, o	0.84
	0:50	S	+, +++++	±, ++, ++++	+, +	+, +++++	±±,	0.91
	1:10	S	++	+, +++	+++	++	o,	0.73
	1:40	S		+	o		o, o	0.88
	2:10	S					o, o	2.11
	2:40	S						2.11
	5:10	D						3.21
Rat group 58 C=1.21 mg./L. T=3.5 min.	0:06	S	++	o o o	o o o o	Sw*, +	o,	1.10
	0:33	S	++	+, ++	o+	Sw, ±, +++	±	1.27
	1:33	S	++	+, ++	+++	+, +++, +++†	o	1.21
	3:03	S		+			o	1.60
	4:33	D					o	3.58
Rat group 59 C=0.082 mg./L. T=57 min.	1:01	S	+++++	+	+++ o +	+++, +++	o,	1.01
	1:12	S	+++++	+, +++++	+, ++++	++++, +++	o	1.40
	1:27	S	+++++	+, ++++	++++	+, ++++, ++++	o	1.03
	1:57	S	+++++	++	o o o o +	++, ++, ++	o	1.18
	2:27	S	+++++	+++	+	++, +++	o	1.26
	2:57	S	+++++	+++	+	++	o	1.46
	3:57	S	++++	++		++, ++	o	1.63
	4:57	D					o	2.59
	5:57						o	2.12
	5:57					†	3.45
	7:00—12:00							4.02

* Sw = Swelling only.
† Beginning "bronchopneumonia" suggested by leukocytic invasion.

TABLE IV

Evaluation of Acute Pathologic Changes in the Lungs of Cats, Rabbits, and Guinea-Pigs after Exposure to an L.D.₅₀₊ of Phosgene.

C-T data	Time after "CG on" (hours)	Sacrificed (S) or died (D)	Emphysema	Congestion of lung	Alveolar edema	Mucosa of bronchioles; swelling, sloughing, and/or plugging	Bronchiolar constriction	Lung/body weight (× 100)
Cats C=0.29 mg./L. T=13 min. "Push-pull" technic	0:16	S	+	o	o	o,+	o	0.74
	0:43	S	++,+	o,+	o	o,++	+	0.99
	1:13	S	++	+,++	+,++	++	+	1.17
	2:13	S	++	++,+++	++	+++	o	1.25
	4:13	S	++	++,++	++,+++	++,+++	o	2.92
	6:28	D	++	++,+++	+++	+++	o	3.35
	8:13	D	++,+	+++	+++	+++	o	3.93
Rabbits *{ C=0.62 mg./L. T=35 min. "Rise-fall" technic	0:40	S	o	+,+	o	o,+	o	0.57
	0:55	S	++	++	o	o,+	o	0.69
	1:03	S	++	++,++	++	+,++	±	0.75
	1:35	D	++	++,+++	++,+++	++,++	o	1.46
	1:46	S	++	+++,++	+++	+,+++	o	1.76
	2:06	D	++	+++	+	++,+++	o	1.56
	2:46		++		+++		o	1.67
Guinea-pigs C=0.34 mg./L. T=9 min. "Push-pull" technic	0:12	S	o	o	o	o	±	0.78
	0:24	S	+	+,+	o	o	+,+	0.81
	0:39	S	++	++,+	o	Sw†	+++	1.18
	1:09	S	++	+,+++	o,+	Sw,+	+	1.13
	2:09	S	++	++,++	++,+++	Sw,++	o,±	1.54
	3:09	S	++	++,++	++,+++	+,+++	o	3.22
	3:39	S	++		+++,++	+,++	o	3.47
	4:24	S		+++,+		+++‡		2.92

* These two rabbits by "push-pull" technic; C × T's were the same.
† Sw = Swelling most prominent.
‡ Beginning "bronchopneumonia" as suggested by leukocytic invasion.

blood vessels became distinguishable first in the capillaries of the alveolar membranes and perhaps the smaller veins. This congestion coincided with, or possibly preceded, the alveolar edema; but definitely followed the changes mentioned above.

Alveolar edema was detected first as a thickening of the septal strands; eosinophilic material in the alveoli was found later. Apparently the fluid distending the septal strands escaped into the alveoli. The tabulated data make it clear that alveolar edema is the last to appear of the several changes of phosgene poisoning.

The lung $\times$ 100/ body weight ratios in the tables are customarily employed as measures of the degree of congestion and edema; they indicate here that edema fluid does not accumulate in appreciable amounts until after the other elements of the "typical" phosgene lung are well developed. The upper limit of normal lung $\times$ 100/body weight ratios for dogs is approximately 1.25; for other species, 1.0 or less.

Neither bronchiolar plugs nor emphysema could be related spatially with alveolar edema and all lesions in this phase tended to be without preferential localization. There is no way of deciding whether the fluid in a given alveolus arose from surrounding or more distant capillaries.

In animals poisoned by smaller doses of phosgene, identical lesions developed in the order outlined, with equally spotty distribution. The rate of development, however, was appreciably slower and the reaction appeared less intense.

No lesions were found in extrapulmonary tissues in this incipient phase.

Histologic evidence of damage to the capillary endothelial cells was never found in these sections (compare Daly *et al.*[13]).

The Critical Phase of Phosgene Poisoning

The anatomic picture in the critical phase is the well known "phosgene-poisoned lung." Therefore, the points noted below are only those which warrant emphasis.

In animals which died during the first 48 hours after exposure, the trachea was usually filled with froth, while the bronchi contained mainly fluid; this finding is related to the abrupt release of as much as 500 cc. of fluid via the trachea immediately before death. The mucosal surfaces of the trachea and larger bronchi were usually smooth, and pale or slightly pink. Rarely was there free fluid in the pleural cavities.

The lungs were large and coarsely mottled with alternating dark red and pale yellowish patches of doughy consistency and only slightly crepitant. The sectioned lung presented a smooth wet surface from

which fluid escaped freely; the contrasting surface patches extended throughout the lung.

The heart was usually dilated, especially the right side, but other than an occasional subendocardial or subepicardial hemorrhage no gross abnormalities were encountered. The liver, spleen, kidneys, intestines, and bone marrow were dusky with congestion. The adrenal glands occasionally showed small hemorrhages in the medulla or at the corticomedullary junction. At times the brain was somewhat hyperemic and edematous; other lesions were not found. Essentially, therefore, the extrapulmonary organs showed congestion with occasional, irregularly distributed focal hemorrhages.

The microscopic pulmonary lesions of this phase are shown in the last few entries of Tables I through IV. The lesions were identical, regardless of species and the varying conditions of exposure, and appear to represent merely full development of the earlier changes. The pathologic evaluation did not always agree completely with the clinical state, since in addition to two instances in the tables, many animals sacrificed before being *in extremis* showed lesions as fully developed as those in animals which had died.

Most alveoli were filled with an eosinophilic granular material presumed to be from the fluid in the unfixed lung (Fig. 6). This was found in alveoli of the usual size, in emphysematously distended alveoli, and in atelectatic areas. The fluid contained fibrin, sometimes in large amounts, which tended to be condensed along the alveolar walls. The alveolar capillaries were dilated and engorged except in the emphysematous areas where they were relatively empty. Small hemorrhages were found but were never a conspicuous feature.

Necrosis and sloughing of the bronchiolar epithelium were now obvious and the bronchioles were clearly dilated (Fig. 7). The mucous membrane of the larger air passages and trachea was relatively intact, but covered with mucus and scattered polymorphonuclear leukocytes. More intense inflammatory lesions were seen in the bronchioles which contained necrotic epithelium, plugs of necrotic tissue, and exuded cells; such bronchioles sometimes formed the center of an inflammatory focus extending into the adjoining parenchyma (Fig. 8). This early pneumonia (pneumonitis) was variable in extent, sometimes being absent. Generally it was focal, but occasionally it was diffuse with scattered cellular and fibrinous exudate.

In animals which survived until the third day, regression of the edema and congestion appeared to have begun, while the cellular exudate frequently increased in severity and extent. In addition to the types of cells previously found, mononuclear phagocytes began to

be prominent in the alveoli. The pneumonia at this time usually had assumed a more or less lobular pattern and was termed bronchopneumonia. The high lung/body weight ratios were maintained.

Microscopic examination of organs other than the lung confirmed the gross finding of congestion and showed occasional tiny hemorrhages. In every instance the lesions were of the types customarily associated with circulatory derangement.

In Table V are summarized the terminal findings in dogs poisoned by oxygen, vagotomized under local anesthesia, or subjected to drugs or vagotomy before or after gassing. These data show first that the phosgene picture is not specific and secondarily that procedures designed to interrupt or facilitate bronchomotor reflexes fail to influence the pathologic changes.

The Regressive and Reparative Phase of Phosgene Poisoning

Data concerning the regressive and reparative phase were obtained from 121 dogs dying or sacrificed between 4 and 138 days after exposure to doses of phosgene which had killed initially between 70 and 99 per cent of the groups they survived. The majority had served as controls in various experiments but some had been used for innocuous therapeutic trials. The dogs were kept in individual indoor kennels on a diet of Purina "checkers," supplemented by cooked horse meat. Although this diet proved to be adequate for normal dogs, inanition was a frequent finding in deaths during the first month after exposure.

Originally an attempt was made to distinguish a regressive stage from a reparative stage, but this was abandoned when it became apparent that: (a) The two processes overlapped not only from animal to animal, but also within various areas of the lungs of one animal. (b) Dogs which made good clinical recoveries showed earlier predominance of reparative processes and more rapid regression of edema, and vice versa. (c) The pneumonic process which had begun in the critical phase varied in degree and persistence; often it obscured the regressive process and probably interfered with repair. In some instances the pneumonic changes were so severe as to appear to be the immediate cause of death. By the third week after exposure, however, the reparative processes predominated, because animals showing little tendency toward repair had died.

Typically, the regressive changes consisted of a gradual diminution of the amount of fluid in the alveoli and reduction of congestion, so that by the end of 2 weeks after gassing the lungs failed to drip fluid on section. Microscopically, the eosinophilic alveolar material took a deeper stain and by the end of the third week had practically disappeared. The trachea and bronchi appeared normal; the mucosa lining

TABLE V

Evaluation of Pathologic Changes in the Lungs of Dogs Subjected to Various Procedures

Dog no.	C.T. and technic (mg. per min. per L.)	Procedure	Sacrificed (S) or died (D)	Time after "CG on" (hours)	Emphysema	Congestion of lung	Alveolar edema	Mucosa of bronchioles; swelling, sloughing, and/or plugging	Bronchiolar constriction	Lung/body weight (×100)
61	14.6	Bilat. vagotomy* before CG	D	2:35	++, +++	+++	+++	++	o, ±	3.98
2	14.6	Bilat. vagotomy* before CG	D	4:35	+++	+++	+++	+++	o	3.95
42	11.0	Bilat. vagotomy* before CG	D	7:13	++	+++	++, +++	++, +++	o	3.47
164	17.9	Bilat. vagotomy* after CG	D	4:40	++	+++	+++	+++, +++‖	o	2.96
161	17.9	Bilat. vagotomy* after CG	D	5:50	+++	+++	+++	+++	o	4.23
44	None	Bilat. vagotomy only	D	22‡	+++	+++	++, +++	+++, +++	o	3.10
45	None	Bilat. vagotomy only	D	23‡	+++	+++	++	++, +++++	o	3.05
50	None	Bilat. vagotomy only	D	12 to 18‡	+++	+++	++	+++	o	3.41
51	None	Bilat. vagotomy only	D	30 to 36‡	+	+++	+	+, +++‖	o	3.04
442	18.6	Atropine† before CG	D	4:39	++	+++	++, +++	+++	o	3.14
643	14.5	Atropine† after CG	S	4:00	+++	+++	+++	+++++‖	o	3.10
798	19.4	Atropine† after CG	D	7:08	+++	+++	+++	++, +++	o	5.02
000	13.7	Eserine† before and after CG	D	6:46	+++	+++	+++	+, +++	o	2.75
909	11.7	Ergotomine† before CG	S	4:14	+++	+++	+, +++	++	o	2.34
913	11.7	Ergotomine† after CG	S	4:01	++	+++	++	++, +++‖	o	1.56
620	None	95+ % O₂ for 48:30 hrs.	D		++	+++	++, +++	++, +++	o	4.09
623	None	95+ % O₂ for 47:00 hrs.	D		+++	+++	+++	++, +++‖	o	3.95
622	None	95+ % O₂ for 48:30 hrs.	D		+++	+++	++, +++	++, +++‖	o	3.56
621	None	95+ % O₂ for 48:30 hrs.	S		o, ±	++	o, +	++	Dilated	1.53

* Performed under procaine anesthesia.
† Drug in dose sufficient to exhibit action.
‡ Time of survival after operation: not gassed.
§ Multiple hemorrhages in alveoli.
‖ Beginning "bronchopneumonia" suggested by leukocytic invasion.

the trachea and bronchi either had sustained no damage, or had regenerated perfectly.

Leukocytic infiltration, or bronchopneumonia, generally became more dense during the first week and changed into foci of consolidation. This reached its peak about 10 to 14 days after exposure in sacrificed animals which probably would have recovered; in those which were obviously moribund the infiltration, in general, was heavier and confluent.

In the gross specimen, elevated, irregular, pale gray patches studded the pleural and cut surfaces of the lung, being more numerous on the former. Beads of purulent material could be expressed from these foci; larger patches had the turgor of early abscesses. Microscopically, the lesions, composed of polymorphonuclear leukocytes, lymphocytes, erythrocytes, and fibrin, resembled a purulent bronchopneumonia (Fig. 9). Generally these lesions were situated in connection with a fine bronchiole, the process spreading distally or laterally to involve varying areas of alveolar tissue. Destruction of alveolar partitions was apparent at times, while in other instances the alveolar walls were infiltrated with monocytes and fibroblasts which extended into the alveolar exudate.

The lung/body weight ratios continued high during this pneumonic process. A return toward normal ratios was associated with resolution of the pneumonia or with minimal infiltration. In other areas of the lung, fibroblastic proliferation was active, forming organized masses which frequently obstructed the bronchioles; sometimes this granulation tissue was found to extend along the airways into alveoli (Fig. 10). During this period the abdominal viscera remained somewhat congested and scattered petechial hemorrhages were still to be found.

It was not possible to establish a relationship between the foci of pneumonia and the early mucosal lesions, although both were essentially bronchiolar. The stained sections of the foci failed to disclose bacteria in the exudate or leukocytes; bacteriologic studies were not carried out.

The fibroblastic proliferation noted above appears to be the means of repair of the alveolar structure since in animals which survived for 1 month after gassing, emphysema, atelectasis, and fibrous scars dominated the picture (Fig. 11). The pneumonic process was still to be found, but it became less and less extensive and active with the passage of time. While the pneumonic foci appeared to heal by scar formation, fibroblastic proliferation also took place in areas free of pneumonia. The bronchiolar epithelium commonly regenerated as cuboid or elongated cells (Fig. 12).

Grossly, the lungs of an animal about 60 days after exposure showed

only emphysema. They offered resistance to sectioning which may be ascribed to condensed fibrous tissue. This scarring, together with scattered emphysema and foci of atelectasis, was seen constantly in the sections. Occasional findings were bronchiectasis, especially in the smaller bronchioles, chronic bronchitis, and tiny pulmonary abscesses.

Various combinations of these lesions persisted in dogs sacrificed more than 6 months after gassing and hence may be regarded as permanent residua of phosgene poisoning. Except for moderate emphysema, scars, and occasional small patches of atelectasis, the lungs were grossly normal. The pleural and bronchial surfaces were smooth. In the sections emphysema and fibrous scarring of the bronchioles were present constantly; small, dense nodules of scar tissue were seen less frequently. Epithelial cells, arranged in single, cuboid, or truly cylindrical layers, had regenerated over or through such scar tissue. Some bronchioles were entirely obliterated by scar tissue, and many alveolar walls were thickened by fibrous connective tissue. Occasionally, small areas of atelectasis containing a few lymphocytes were encountered. The most prominent constant change was distention and fragmentation of alveoli into irregularly enlarged chambers; this may be termed chronic lobular emphysema. It is important, however, that even in lungs with the most extensive residua, there were large amounts of apparently normal alveolar tissue.

SUMMARY OF THE ANATOMIC CHANGES FOLLOWING EXPOSURE TO PHOSGENE

Emphysema, lobular in distribution, was found constantly in animals sacrificed at once after exposure to doses which killed between 70 to 99 per cent of the animals. An equally constant, but less obvious concurrent finding was sloughing of the bronchiolar mucosa with formation of intraluminal plugs. Shortly thereafter there developed perivascular and peribronchial edema which preceded the alveolar edema. Bronchiolar constriction was found regularly in only one of the several species examined and even in this species constriction was soon supplanted by dilatation.

These changes gradually but steadily progressed, producing finally the typical lung of phosgene poisoning. The alveolar edema contained a high content of protein which by analysis was approximately equal to plasma protein levels.[12,14] Leukocytic infiltration, particularly in the bronchiolar regions, began during the early part of this critical phase and became more prominent the longer the animal survived. Frequently, during regression of the congestion and edema, the infiltration gave rise to a picture almost identical with bronchopneumonia.

In animals with the least pneumonitis, repair of the damage was

most rapid and widespread, but even where infiltration was so extensive as to be considered the immediate cause of death, some evidence of repair was found. Repair consisted of fibroblastic ingrowth which finally gave rise to scar tissue; the process apparently proceeded regardless of complicating pneumonitis. The bronchiolar surfaces were relined by cuboid or cylindrical epithelium.

The lungs of clinically recovered animals showed residua consisting chiefly of foci of scar tissue and lobular emphysema, but tiny patches of atelectasis and cellular infiltration occasionally were still present.

Changes in tissues other than the lungs resembled closely those seen in cardiovascular disease, and had no unique characteristics. Petechial hemorrhages were found frequently but were inconstant in distribution and extent. Evidence of primary damage to the heart and central nervous system was lacking.

DISCUSSION

Since there are no data on the lesions in man during the incipient or developmental phase, it is impossible to compare these changes in man and animals. Tentatively, it may be assumed that man would respond like several other species and show a comparable sequence of lesions. The findings reported above are in essential agreement with three recent experimental studies,[15-17] although differences in emphasis on predominance and rate of development of the various lesions are to be found. These differences seem minor, however, in view of variations in histologic technic (dissecting microscope, vital staining) and variation in gassing methods and concentrations. The older reports on animals sacrificed sporadically in this phase provide inadequate information.[2,11,18]

The findings in man during the critical phase are in general similar to those in experimental animals, but certain inconsistencies warrant closer comparison. In the Hamburg disaster,[19] in which pure phosgene escaped, the pulmonary findings were almost identical with those described in our animals, although the cellular inflammatory lesions appeared earlier and the fibrin later than is usual in experimental poisoning. Outside the lung, however, there were found lesions which seldom or never have been recorded in animals: Focal necrosis of the adrenals, thrombosis of large vessels, subarachnoid hemorrhages, cellular degeneration of the gray matter and a generalized hyperemia, said to extend only to the white matter. Other observers have reported "ring-hemorrhages" in the brain.[6,20,21] In contrast, the autopsy reports on two men who died 19 and 22 hours after accidental exposure[22] stressed edema, emphysema, cardiac dilatation, and necrosis with

sloughing of the laryngeal, tracheal, and bronchial mucosa. This extra-pulmonary sloughing suggests exposure to extraordinarily high concentrations of phosgene, but death was not sufficiently prompt to bear this out.

The autopsy reports of 105 soldiers of World War I,[6] who were reputedly gassed with phosgene, contain records of pleural effusion; this also has been recorded by others.[23,24] Effusion was noted rarely in dogs and cats, but it was not uncommon in rodents when autopsy was delayed. Other frequent findings were subendocardial and subpericardial hemorrhages, thrombosis of the pulmonary vessels with thrombi attached to the cardiac valves, and edema and extreme congestion of the brain. Hemorrhages in various parts of the central nervous system were seen only in men who died 36 hours or more after exposure. "Bronchopneumonia" was stated to have been present as early as 12 hours after exposure.[6]

These examples are representative of the pathologic details in the literature on phosgene poisoning in man in the acute stage.[1,4,5,18-21,23-28] In general, the pulmonary lesions do not differ essentially from those recorded on animals poisoned under controlled conditions; [2,11,17,18,29,30] the variations in detail and emphasis are probably attributable in large part to the factors outlined in the introduction. The extrapulmonary lesions, with the exception of congestion, seem much more prominent in man than in animals, but it is questionable whether this constitutes a real difference in the reaction to phosgene. Because the extrapulmonary lesions resemble those found in death from circulatory failure, and since circulatory insufficiency from blood volume loss is present in phosgene poisoning, it is illogical to postulate the formation *in vivo* of specific humoral toxic agents which might cause these lesions.[6,17,20]

Records of thrombosis or other obstructions to the pulmonary circulation in both man and animals in the older literature [6,11,31,32] have no counterpart in the data reported here and the reason for this discrepancy is not known. In any event, it is probable that pulmonary obstruction has little if any significance in the pathogenesis of this edema, since, in addition to the negative evidence, we have found that completely heparinized animals follow the typical phosgene course, with the same sequence of changes.[12]

Autopsy data on man in the *regressive and reparative phase* are fragmentary and described mainly in terms of "bronchopneumonia." [6,11,33] In a man who died 11½ days after exposure [10] the combination of fibroblastic proliferation, macrophages in the alveoli, chronic bronchitis and bronchiectasis, and residual edema is almost

identical with that in dogs which died at a comparable interval after gassing. Also the findings in a man who died 3 months after exposure [34] are very similar to those in dogs dying about 21 days after exposure. Thus it is reasonable to assume that the reparative processes in human survivors take the same course that has been described for dogs. Evidence corroborating this view is the pulmonary fibrosis, chronic bronchial inflammation, and emphysema detected by x-ray and physical examination.[23,35-38]

It will be noted that the concentration of phosgene may be varied reciprocally with duration of exposure by a factor of 10, without detectably affecting the pathologic and physiologic findings. The data bear out the assumptions on which the dosage system is based, which in turn is the basis for a systematic experimental study of phosgene poisoning. The range of reciprocity is probably much greater than 10.

According to the above data, certain physiologic and clinical concepts of phosgene poisoning need revision. The "latent" period of phosgene poisoning, the more or less asymptomatic interval between exposure and the onset of detectable edema, is clearly a fallacy since the anatomic pulmonary damage, begun during gassing, steadily progresses. The clinical problem in phosgene poisoning (and similar toxic agents) must lie in the application of measures designed to minimize its progress. Clearly the time for treatment is before, not after, edema has appeared, but because such therapy is unknown, one is confined to providing whatever symptomatic relief is indicated.

We never have predicted successfully the probable outcome from clinical observations alone. Dogs poisoned by doses of phosgene killing 70 to 99 per cent of the group immediately showed bradycardia, a general lassitude, motor activity of the colon and bladder, a flared chest, and a rapid shallow type of restricted breathing; there was no relation between the severity of these signs and survival. Later when râles could be heard, the tachycardia, leaden cyanosis, asthmatoid breathing, and prostration were merely confirmatory evidence of the degree of involvement of the lungs. However, because the edema fluid is practically plasma, it was possible to estimate the rate of development and ultimate degree of edema by frequent hematocrit determinations. In general it was observed that the slower the onset and rate of hemoconcentration, or the longer the interval between gassing and clinically detectable edema, the better was the prognosis.

The question of bronchoconstriction, or bronchiolar constriction, in phosgene poisoning needs re-examination. In the past, assumption of this condition was based on subjective substernal constriction and similarity of the breathing to that in asthma.[5] Histologic evidence support-

ing this [1,11] was not qualified with regard to the fact that the fixed tissue might not represent the living state. The present data, thus qualified, suggest that constriction, if present, is transitory and not significant in the critical phase of poisoning when the breathing is most analogous to that of asthma. It may be important that in the one species, the guinea-pig, in which histologic bronchoconstriction was seen, the bronchial smooth muscle is arranged like that in man.[39] Experiments on dogs and rats [12] have led to the conclusion that the asthmatoid breathing is not of constrictor origin, since: (a) In hot weather panting may alternate with periods of asthmatoid breathing. (b) Administration of CO_2 immediately converts the breathing into free, rapid, deep movements. (c) Pressure on the larynx removes the expiratory grunt without shortening the expiratory effort. (d) None of the sympathomimetic, parasympatholytic, or myotropic antispasmodic drugs which are effective in asthma exerted more than a transitory effect on breathing. Unequivocal evidence of relaxation of bronchospasm was not seen. Eserine did not exaggerate the asthmatoid breathing, and vagotomy before or after gassing resulted in breathing characteristic of the ungassed animal, slow and deep with prolonged inspiration. Drugs which act on the heart peripherally (and vagotomy) readily modified the reflex bradycardia without materially changing the breathing. The data in Table V show that the characteristic findings were unchanged by the two procedures most likely to accent or minimize reflex bronchoconstriction. The evidence obtained from gassed lung preparations designed to demonstrate bronchoconstriction is equivocal.[2,13,15]

Nevertheless, evidence of subnormal ventilation is found in the transitory arterial anoxemia and the CO_2 retention and respiratory acidosis seen during the incipient phase.[12,16] Two possible causes of this, other than bronchoconstriction, are (a) obstruction of the airways by the plugs of sloughed epithelium and (b) edema of the remaining bronchiolar epithelium, which is supplied by the bronchial artery, in connection with which the earliest edema was noted. The intrabronchial emboli have been observed before [11,18] but were not assigned a significant rôle. Their occurrence, synchronous with the emphysema, suggests a causal relation which has not been demonstrated. In the gassed dog-lung preparation desquamation of the bronchial mucosa has been observed, although bronchial emboli were not described.[13] Experiments with balloon models indicated that the alveoli supplied by unblocked bronchioles are those which would stretch and rupture under negative pressure.[12] It is tempting to postulate that sloughing of the bronchiolar mucous membrane with plugging of the lumen

causes the unbarricaded alveoli to rupture as they follow the expansion of the chest, which is induced during gassing and may be maintained reflexly. When sufficient edema is present another form of bronchial obstruction probably develops which nullifies all inhalational therapy. As the edema fluid moves about via the bronchial system it mixes with air to form a moderately stiff foam which produces a water lock proximal to otherwise functional alveoli.

In the healing process some bronchial plugs are recanalized, but if they should be replaced by scar tissue the result would be atelectasis, as was observed. The emphysema seems to persist much as it first appeared and it is doubtful that such areas are functionally active. This "vesicular" emphysema has been described as a common aftermath of poisoning by any of the pulmonary irritant gases and it has been implicated in the etiology of the "effort syndrome" found in gas casualties.[23,37,40-42] Psychic factors have been assigned the major rôle in this condition [43] but abnormalities of pulmonary ventilation recently have been detected.[44]

It is improbable that the plugs and emphysema bear a causal relation to the edema, chiefly because in exposure to very low phosgene concentrations edema appears without emphysema or significant bronchiolar changes.[3,45] The hemodynamics of the pulmonary circulation are not grossly disturbed in phosgene poisoning [46,47] and so it must be assumed that the capillaries are damaged functionally by the gas. It is obvious, therefore, that this study has merely reaffirmed that available histologic methods are inadequate to demonstrate the abnormal functional state of the capillary endothelium. Capillary congestion does precede the alveolar edema, but there is no reason to assume that congestion causes loss of normal permeability. Because the hemoconcentration was arrested and reversed in dogs 15 to 35 hours after exposure, it is probable that restoration of normal capillary permeability began in that period.

The majority of dogs poisoned with the doses used in these experiments died of respiratory failure before or near the peak of hemoconcentration. The type of death indicates functional disintegration of the central nervous system, prior to that of other essential organs such as the heart. Since movement of plasma into the alveoli is responsible for both the major defects, the anoxic anoxia and the shock-like hemodynamics, it seems beside the point to insist that one or the other of these two secondary features is the more lethal. Moreover, both these defects set up vicious cycles which are interlocking and supplementary. It is of interest, however, that the rabbit, a "wet" animal, dies of

typical pulmonary edema without significant hemoconcentration, and therefore without stagnant anoxia.[12,14]

The resorption of edema fluid appears to take place slowly, presumably because of its high protein content.[48-50] Some fluid is removed by the lymphatics [14,30,51] but the chief mode of regression of the edema seems to be degradation of the protein into diffusible molecules by the macrophages and leukocytes, which were observed to migrate in. It is still not clear whether this inflammatory reaction is of bacterial origin or is a further manifestation of the original chemical damage by phosgene. The different susceptibility of the lungs of dog and man to bacterial invasion warrants caution in extending these interpretations of the inflammatory reaction to man.

In addition to failure of pulmonary function due to residual edema and infiltration, death in the early reparative phase may result from functional failure of key organs such as the kidney, which have been devitalized by the previous prolonged anoxia.[52,53] A few dogs in which death was delayed for 3 or 4 weeks after exposure showed inanition clinically in conjunction with an apparently more gradual progression of the inflammatory response.

This healing process seems to consist of regeneration of a more or less normal epithelial layer and scar tissue formation, regardless of whether the initial damage had progressed into a focal or confluent inflammatory reaction. This method of healing and the residua are common to lungs poisoned by phosgene, chlorpicrin, lewisite, and mustard vapor.[17,54,56] Evidence of a type reaction on the part of the lung is seen also in the fact that the acute lesions of phosgene poisoning are identical in many respects with the acute lung damage produced by exposure to chlorine and chlorpicrin,[11] nitric oxides,[55,56] ketene,[15] high oxygen tensions, and following deep x-ray therapy,[57] bilateral vagotomy,[58] anaphylaxis in certain species,[59] and the hot gasses of the Cocoanut Grove fire.[60]

SUMMARY

1. The pathologic anatomy of dogs and other species was investigated after experimental exposure to median doses of phosgene. Particular attention was placed on the sequence of development of the lesions and on the anatomic changes found in animals that survived the acute or critical phase in which the majority died.

2. Extensive emphysema, sloughing of the bronchiolar mucosa, and questionable bronchiolar constriction were found at once after gassing. Peribronchial edema, pulmonary congestion, and alveolar edema de-

veloped subsequently and in that order. The rapidity of development and extensiveness of these lesions were roughly proportional to the severity of the exposure.

3. Recovery from the massively edematous lung of "typical" phosgene poisoning was found to be primarily a process of resorption of edema and scarring. A moderate cellular inflammatory reaction accompanied this process, but sometimes it became so excessive as to be indistinguishable from bronchopneumonia.

4. The residua of phosgene poisoning consisted of pulmonary scarring, lobular emphysema, and small, irregular areas of atelectasis and bronchitis.

5. Comparison of the data with the scanty and somewhat unsatisfactory data on man leads to the conclusion that the processes in man and animals appear to be similar.

6. The findings reported here indicate that certain present concepts of phosgene poisoning need revision.

7. Study of the pathologic anatomy of phosgene leads to the view that the response of the lung to phosgene is a type reaction common to several other agents.

REFERENCES

1. Chemical Warfare Medical Committee, Report no. 1. Notes on the Pathology and Treatment of the Effects of Pulmonary Irritant Gases. His Majesty's Stationery Office, London, March, 1918, 20 pp.

2. Laqueur, E., and Magnus, R. Über Kampfgasvergiftungen. III. Experimentelle Pathologie der Phosgenvergiftungen. *Ztschr. f. d. ges. exper. Med.,* 1921, 13, 31–179.

3. Wirth, W. Über die Wirkung kleinster Phosgenmengen. *Arch. f. exper. Path. u. Pharmakol.,* 1936, 181, 198–206.

4. Chemical Warfare Medical Committee, Report no. 3. The Symptoms and Treatment of the Late Effects of Gas Poisoning. His Majesty's Stationery Office, London, April, 1918, 9 pp.

5. Gilchrist, H. L. Symptoms and Treatment. In: The Medical Department of the United States Army in the World War. Government Printing Office, Washington, D.C., 1926, 14, 250–271.

6. Groll, H. Anatomische Befunde bei Vergiftung mit Phosgen (Kampfgasvergiftungen). *Virchows Arch. f. path. Anat.,* 1921, 231, 480–518.

7. Armstrong, H. G. Principles and Practice of Aviation Medicine. Williams & Wilkins Co., Baltimore, 1943, ed. 2.

8. Hailman, H. F. Lowest barometric pressure compatible with life in an atmosphere of 100 per cent oxygen. *Proc. Soc. Exper. Biol. & Med.,* 1943, 53, 221–222.

9. Van Liere, E. J. Anoxia: Its Effect on the Body. University of Chicago Press, Chicago, 1942, 269 pp.

10. Flury, F. Über Kampfgasvergiftungen. I. Über Reizgase. *Ztschr. f. d. ges. exper. Med.,* 1921, 13, 1–15.

11. Winternitz, M. C. Collected Studies on the Pathology of War Gas Poisoning. Yale University Press, New Haven, 1920, pp. 35–66.

12. Unpublished observations, 1944.

13. Daly, I. de B., Eggleton, P., Elsden, S. R., and Hebb, C. O. A biochemical study of isolated perfused lungs with special reference to the effects of phosgene. *Quart. J. Exper. Physiol.*, 1944-46, **33**, 215–240.

14. Cameron, G. R., and Courtice, F. C. The production and removal of oedema fluid in the lung after exposure to carbonyl chloride (phosgene). *J. Physiol.*, 1946, **105**, 175–185.

15. Lushbaugh, C. C., Tobias, J. M., Patt, H. M., Postel, S., Swift, M. N., and Gerard, R. W. Personal communication, 1944.

16. Bunting, H. *et al.* Personal communication, 1944.

17. Rothlin, E. Pathogénie et thérapeutique de l'intoxication par le phosgène. *Schweiz. med. Wchnschr.*, 1941, **22**, 1526–1535.

18. Chemical Warfare Medical Committee, Report no. 2. The Histological Effects Produced by Gas Poisoning and Their Significance. His Majesty's Stationery Office, London, April, 1918, pp. 4–36.

19. Wohlwill, F. Ueber eine Massenvergiftung durch Phosgengas in Hamburg; zur pathologischen Anatomie der Phosgenvergiftung. *Deutsche med. Wchnschr.*, 1928, **54**, 1553–1557.

20. Adelheim, R. Beiträge zur pathologischen Anatomie und Pathogenese der Kampfgasvergiftung. *Virchows Arch. f. path. Anat.*, 1922, **236**, 309–360. *Ibid.*, 1923, **240**, 417–440.

21. Mott, F. W. Punctiform haemorrhages of the brain in gas poisoning. *Brit. M. J.*, 1917, **1**, 637–641.

22. Delépine, S. Summary of notes on two fatalities due to inhaling phosgene (COCl₂). *J. Indust. Hyg. & Toxicol.*, 1922-23, **4**, 433–440.

23. Gilchrist, H. L., and Matz, P. B. The residual effects of warfare gases: the use of phosgene gas, with report of cases. *M. Bull. Vet. Admin.*, 1933-34, **10**, 1–36.

24. Wilder, R. H. Fifty Necropsies of Phosgene Cases. Historical Division, Surgeon General's Office, Washington, D.C.

25. Gilchrist, H. L. Reports on the After Effects of Warfare Gases. War Department, Chemical Warfare Service, Medical Division, Washington, D.C., 1922, 112 pp.

26. Pappenheimer, A. M. Pathological Action of War Gases. In: The Medical Department of the United States Army in the World War. Government Printing Office, Washington, D.C., 1926, **14**, 87–249.

27. Gerber, I. Un cas d'intoxication mortelle par le phosgène. *Rev. méd. de la Suisse Rom.*, 1920, **40**, 356–362.

28. Roos, A. Phosgenvergiftungen. *Vrtljschr. f. gerichtl. Med.*, 1914, **48**, 67–101.

29. Claussen. Zur pathologischen Anatomie und Histologie der Phosgenvergiftung bei Tieren. *Ztschr. f. Infektionskr.*, 1931, **40**, 1–18.

30. Rothlin, E. Experimenteller Beitrag zur Pathologie und Therapie der Spätfolgen des durch Phosgen erzeugten Lungenödems. *Schweiz. med. Wchnschr.*, 1940, **21**, 641–647.

31. Dunn, J. S. The effects of multiple pulmonary embolism of pulmonary arterioles. *Quart. J. Med.*, 1919-20, **13**, 129–147.

32. Meek, W. J., and Eyster, J. A. E. Experiments on the pathological physiology of acute phosgene poisoning. *Am. J. Physiol.*, 1920, **51**, 303–320.

33. Gilchrist, H. L., and Poore, J. E. Statistical Consideration of Gas Casualties. In: The Medical Department of the United States Army in the World War. Government Printing Office, Washington, D.C., 1926, 14, 273–293.

34. Miller, J. W. Über die pathologische Anatomie des Spättodes nach Kampfgas-(Perstoff–)Vergiftung. *Beitr. z. path. Anat. u. z. allg. Path.*, 1923–24, **72**, 339–343.

35. Abbott, W. N. The incidence of pulmonary disease following exposure to vesicant and asphyxiating gases. *Brit. M. J.*, 1933, 2, 862–865.

36. Hankins, J. L., and Klotz, W. C. Permanent pulmonary effects of gas in warfare. *Am. Rev. Tuberc.*, 1922–23, 6, 571–574.

37. Mearns, A. Chronic effects of inhalation of suffocating gases. *J. Roy. Army M. Corps*, 1922, 38, 350–357.

38. Bonnamour, S., and Badolle, A. La dilatation bronchique des gazés. Sa mise en évidence par le lipiodol. *Bull. Acad. d. méd., Paris*, 1928, 99, 235–237.

39. Miller, W. S. The Lung. C. C. Thomas, Springfield, Ill., 1937, 209 pp.

40. Chemical Warfare Medical Committee, Report no. 5. The Reflex Restriction of Respiration after Gas Poisoning. His Majesty's Stationery Office, London, April, 1918, 10 pp.

41. Berghoff, R. S. The more common gases; their effect on the respiratory tract. Observation of two thousand cases. *Arch. Int. Med.*, 1919, 24, 678–684.

42. Staehelin, R. Die Spätfolgen der Vergiftungen durch Kampfgase für die Respirationsorgane. *Jahresk. f. ärztl. Fortbild.*, 1920, 11, no. 2, 17–24.

43. Rennie, T. A. C., and Small, S. M. Psychological Aspects of Chemical Warfare. Josiah Macy Jr. Foundation, Review Series, 1943, vol. 1, no. 1.

44. Gaidston, M., and Luetscher, J. A., Jr. Oxygen and carbon dioxide tensions in arterial blood and alveolar air at rest and after exercise in healthy subjects and in patients exposed to phosgene. *Federation Proc.*, 1946, 5, 31.

45. Cameron, G. R. Personal communication, 1944.

46. Gibbon, M. H., Bruner, H. D., Boche, R. D., and Lockwood, J. S. Studies on experimental phosgene poisoning. II. Pulmonary artery pressure in phosgene poisoned cats. 1946. To be published.

47. Patt, H. M., Tobias, J. M., Swift, M. N., Postel, S., and Gerard, R. W. Hemodynamics in pulmonary irritant poisoning. *Am. J. Physiol.*, 1946, 147, 329–339.

48. Fox, J. P. The permeability of the lungs to antibodies. *J. Immunol.*, 1936, 31, 7–23.

49. Jones, F. S. The permeability of the lining of the lower respiratory tract for antibodies. *J. Exper. Med.*, 1924, 40, 73–79.

50. Drinker, C. K., Warren, M. F., and MacLanahan, M. The absorption of protein solutions from the pulmonary alveoli. *J. Exper. Med.*, 1937, 66, 449–458.

51. Drinker, C. K. Pulmonary Edema and Inflammation. Harvard University Monographs in Medicine and Public Health, no. 7. Harvard University Press, Cambridge, 1945.

52. Phillips, R. A., Dole, V. P., Hamilton, P. B., Emerson, K., Jr., Archibald, R. M., and Van Slyke, D. D. Effects of acute hemorrhagic and traumatic shock on renal function of dogs. *Am. J. Physiol.*, 1946, 145, 314–336.

53. Shorr, E., Zweifach, B. W., and Furchgott, R. F. On the occurrence, sites and modes of origin and destruction, of principles affecting the compensatory vascular mechanisms in experimental shock. *Science*, 1945, 102, 489–498.

54. Koontz, A. R. War gases and tuberculosis: an experimental study. *Arch. Int. Med.*, 1927, 39, 833–864.

55. Fleming, A. J. A method for handling cases gassed with nitrous fumes— including the diagnostic and prognostic value of blood platelet counts in assessing pulmonary irritation. *Indust. Med.*, 1943, 12, 127–132.

56. von Oettingen, W. F. The Toxicity and Potential Dangers of Nitrous Fumes. Public Health Bulletin no. 272. Government Printing Office, Washington, D.C., 1941, 34 pp.

57. Boys, F., and Harris, I. D. The effect of heparinization on experimental post-irradiation tissue changes in the lung. A preliminary study. *Am. J. Roentgenol.*, 1943, 50, 1–8.

58. Weiser, J. Zur Frage der sog. Vaguspneumonie. *Arch. f. d. ges. Physiol.*, 1932–33, 231, 68–76.

59. Moon, V. H. The pathology and mechanism of anaphylaxis. *Ann. Int. Med.*, 1938–39, 12, 205–216.

60. Mallory, T. B., and Brickley, W. J. Symposium on the management of the Cocoanut Grove burns at the Massachusetts General Hospital: Pathology: with special reference to the pulmonary lesions. *Ann. Surg.*, 1943, 117, 865–884.

[Illustrations follow]

DESCRIPTION OF PLATES

PLATE 151

FIG. 1. Emphysema in the lung of a dog, showing thinning of the alveolar septa. Animal sacrificed 38 minutes after beginning of exposure to phosgene; Concentration of phosgene (C) = 0.49 mg. per liter of air. Duration of exposure (T) = 30 minutes. × 92.

FIG. 2. Bronchiolar constriction. The mucosa is compressed and the lumen is obviously narrowed. Dog sacrificed 8 minutes after beginning of exposure. C = 2.98 mg. per liter; T = 3 minutes. × 92.

1

2
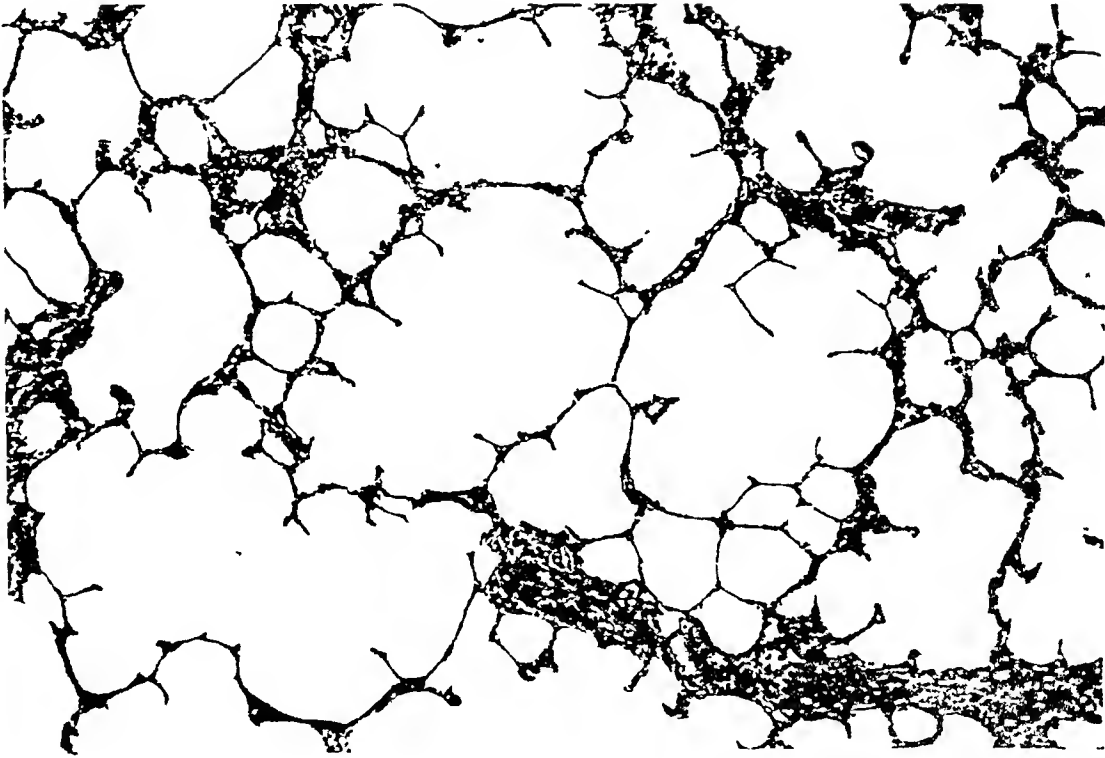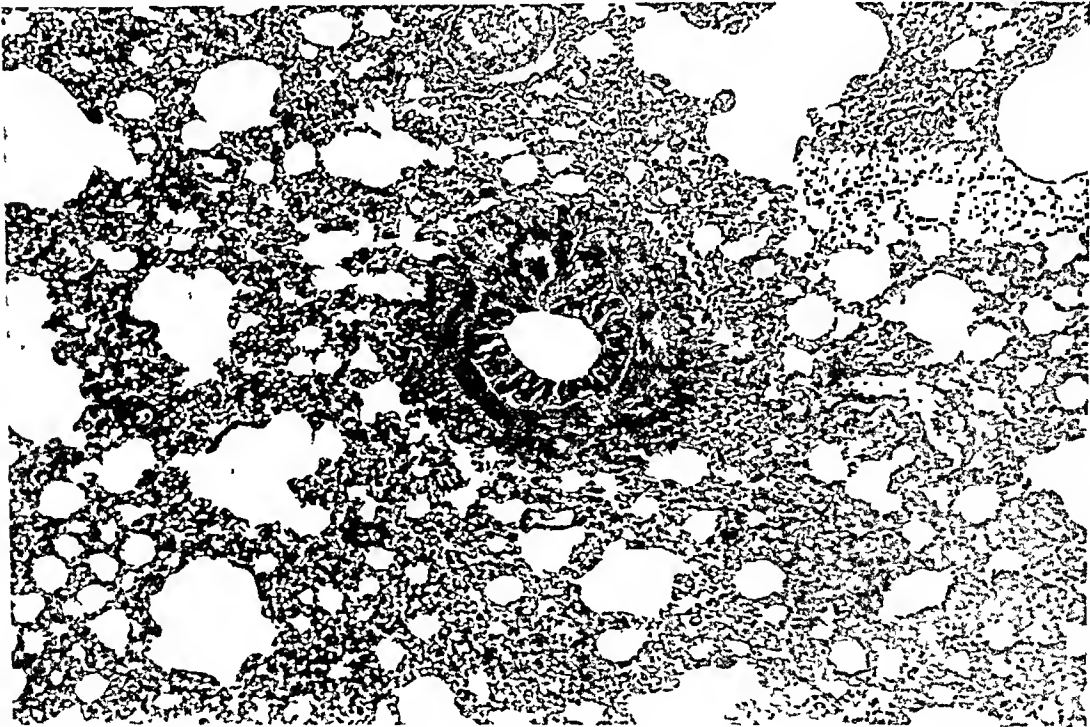
Coman, Bruner, Horn, Friedman, *et al.*   Experimental Phosgene Poisoning

PLATE 152

FIG. 3. Widely dilated bronchiole, found in later stage. The mucosa has sloughed away and the surrounding alveoli are edematous. Dog sacrificed 4 hours and 10 minutes after beginning of exposure. C = 3.33 mg. per liter; T = 3 minutes. × 92.

FIG. 4. Bronchiole containing a plug of desquamated epithelium which has been transported from elsewhere, since the bronchiolar mucosa at this point is still intact. Dog sacrificed 1 hour after beginning of exposure. C = 2.98 mg. per liter; T = 3 minutes. × 200.

3
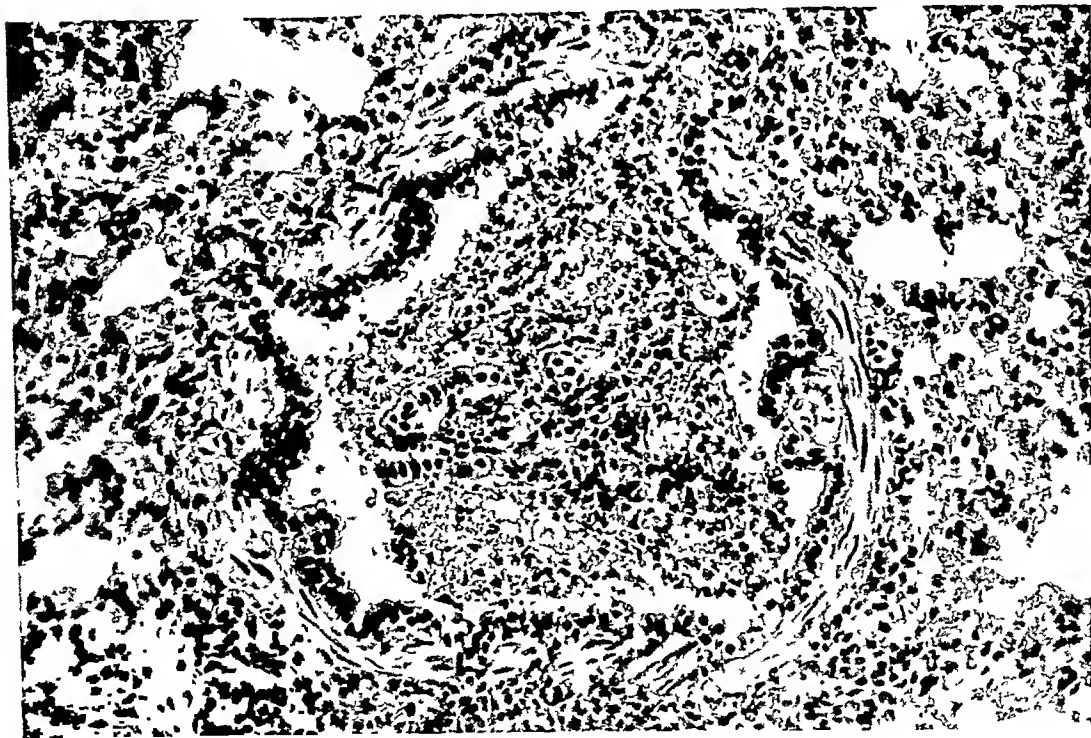
4

Coman, Bruner, Horn, Friedman, *et al.*

Experimental Phosgene Poisoning

FIG. 5. A wide lace-like cuff surrounding a vessel, indicative of perivascular edema. The adjacent alveoli contain no fluid. Dog sacrificed 2 hours and 4 minutes after exposure. $C = 3.30$ mg. per liter; $T = 3$ minutes. $\times$ 20.

FIG. 6. Typical alveolar edema in the critical phase. Of note is the patchy emphysema. Dog died 7 hours after exposure. $C = 3.22$ mg. per liter; $T = 3\frac{1}{2}$ minutes. $\times$ 92.

5
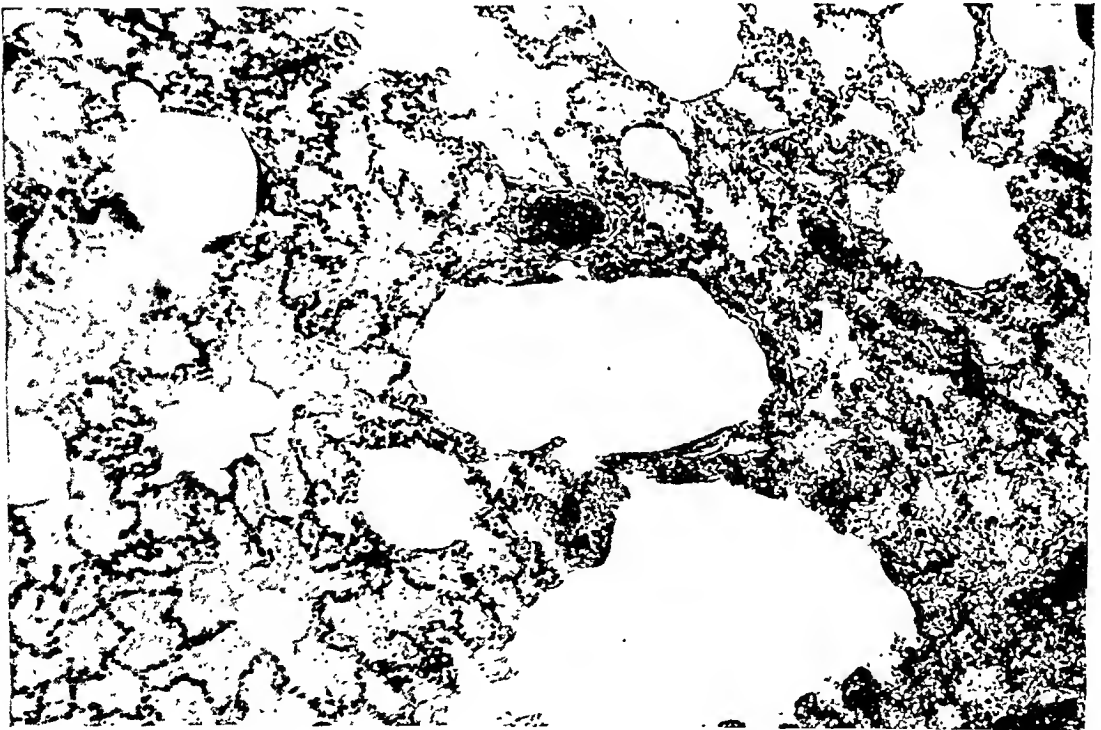
6

Coman, Bruner, Horn, Friedman, *et al.*
Experimental Phosgene Poisoning

PLATE 154

FIG. 7. A widely dilated bronchus from which the mucosa has sloughed, together with alveolar edema and emphysema, in the critical phase of phosgene poisoning. Dog died 8 hours and 17 minutes after exposure. C = 0.759 mg. per liter; T = 30 minutes. × 92.

FIG. 8. A denuded bronchiole containing polymorphonuclear leukocytes which forms the center of an inflammatory focus extending into the surrounding alveoli. The exudate consists of fibrin and scattered polymorphonuclear leukocytes and lymphocytes. Dog died 12½ hours after exposure. C = 0.263 mg. per liter; T = 30 minutes. × 180.

PLATE 154

7

8
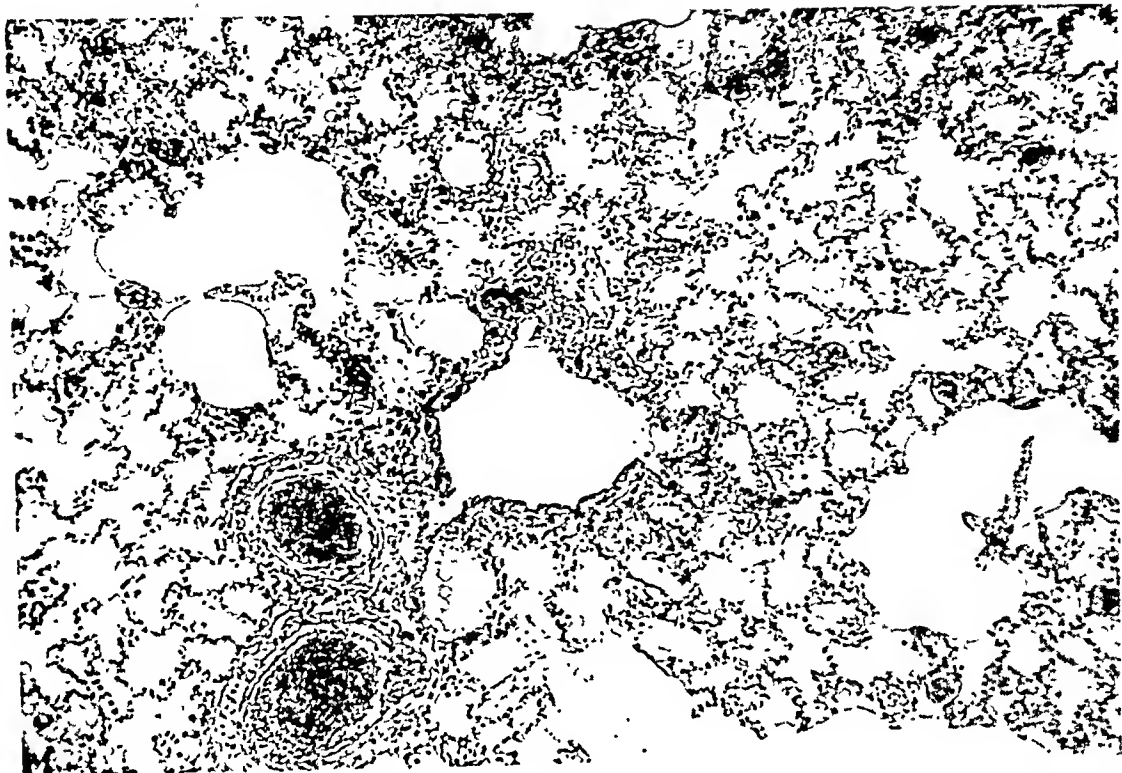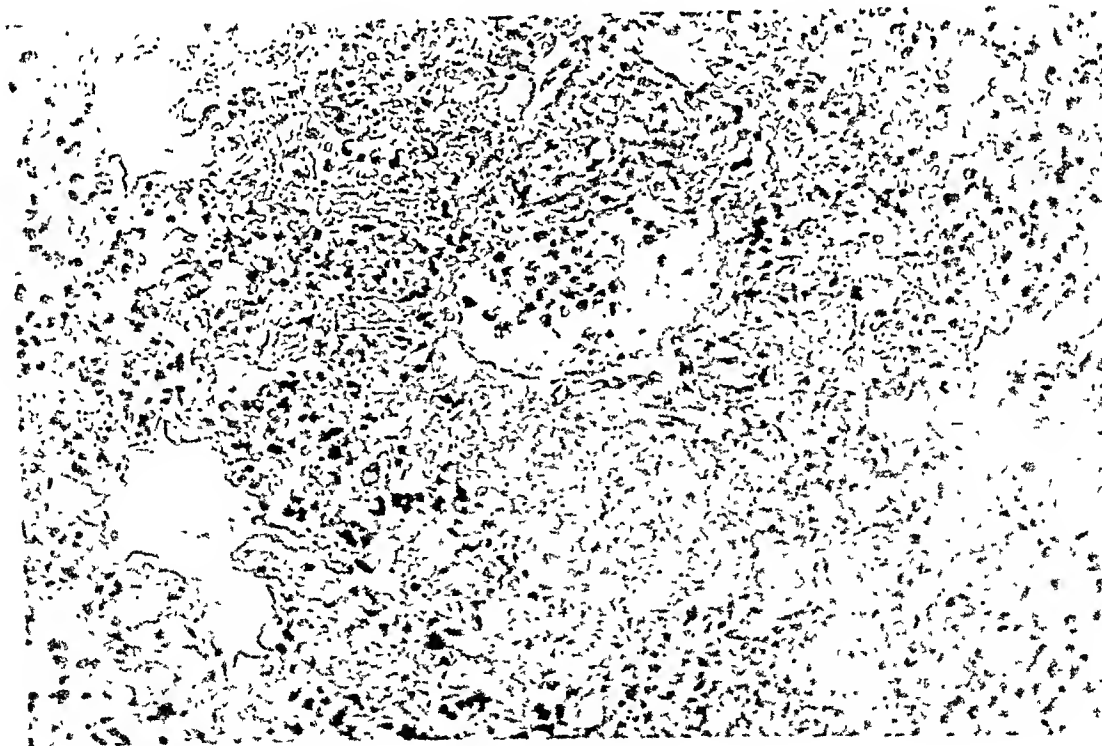
FIG. 9. Advanced bronchopneumonia in the lung of a dog dying 20 days after exposure to phosgene. The alveoli are filled with polymorphonuclear leukocytes, lymphocytes, fibrin, and a few red cells. $C = 0.238$ mg. per liter; $T = 30$ minutes. $\times$ 180.

FIG. 10. Granulation tissue replacing alveoli in the lung of a dog that died 72 hours after exposure. $C = 0.264$ mg. per liter; $T = 30$ minutes. $\times$ 300.
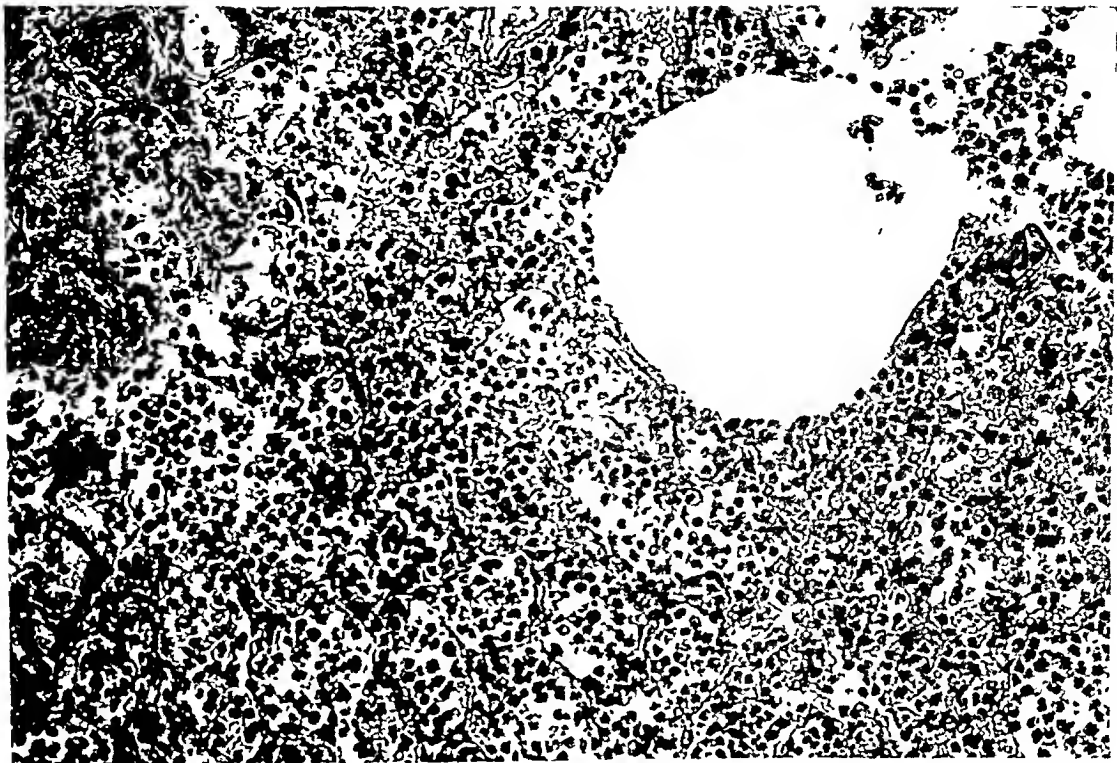
9
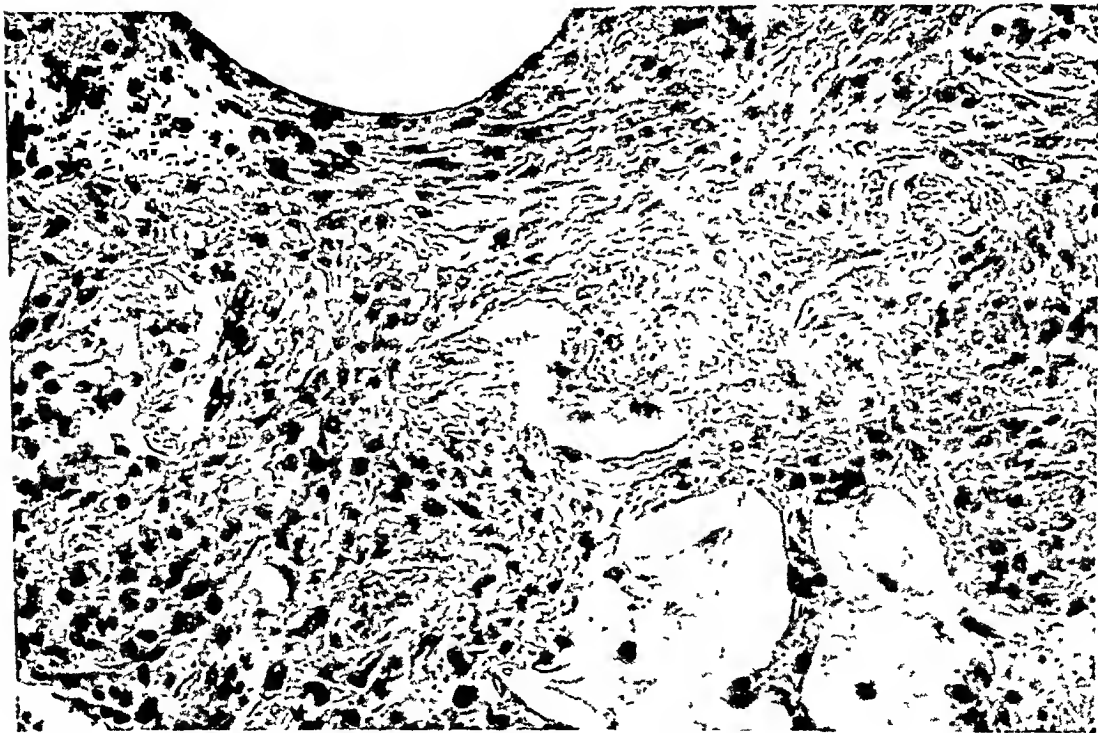
10

Coman, Bruner, Horn, Friedman, *et al.*

Experimental Phosgene Poisoning

PLATE 156

FIG. 11. Section of the lung of a dog sacrificed 27 days after exposure to phosgene. The emphysema persists and parts of the lung are atelectatic; patches of fibrosis are apparent. C = 0.295 mg. per liter; T = 30 minutes. × 83.

FIG. 12. Regeneration of bronchiolar mucosa. The lining cells are cuboidal in some areas, elongated in others. Dog died 6½ days after exposure to phosgene. C = 0.295 mg. per liter; T = 30 minutes. × 300.
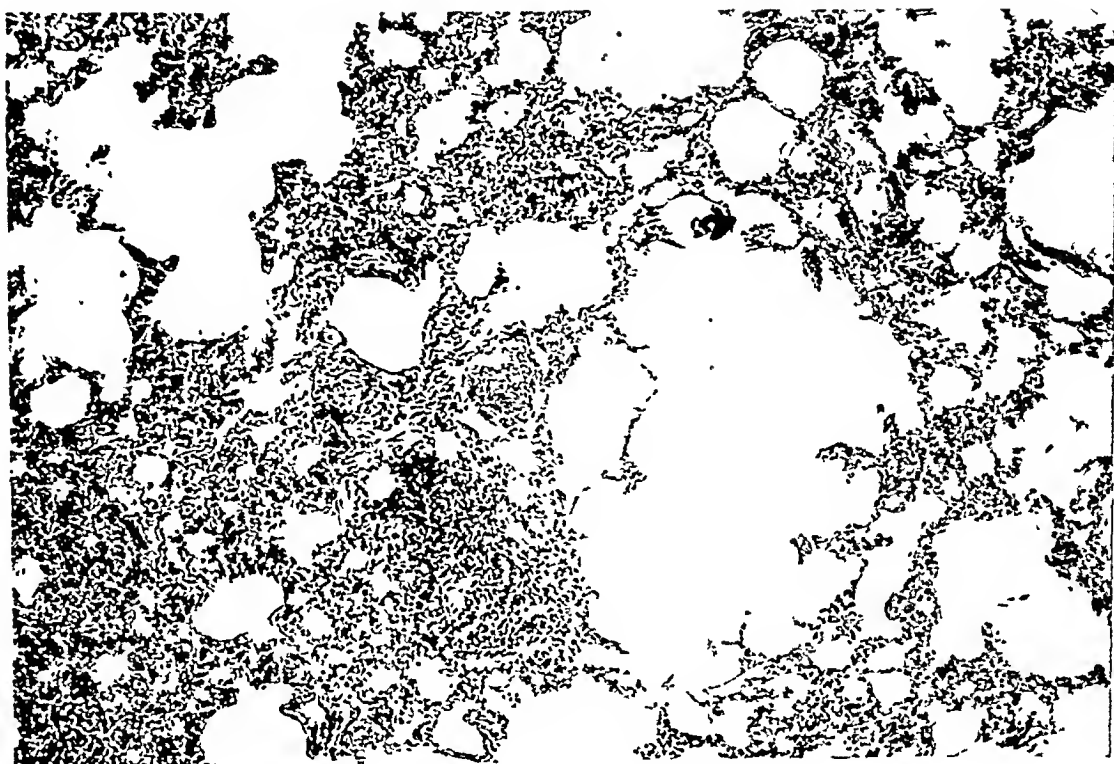
11

12
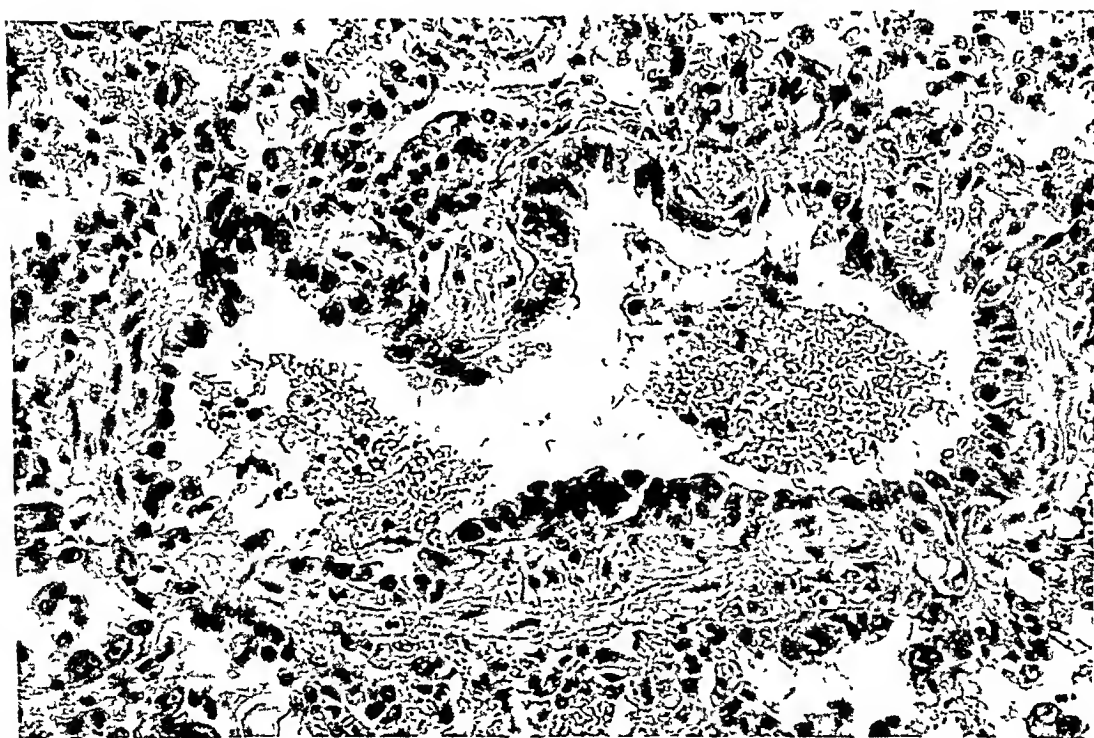
Coman, Bruner, Horn, Friedman, *et al.*

Experimental Phosgene Poisoning

THE PATHOGENESIS OF CONGENITAL POLYCYSTIC LUNG AND ITS CORRELATION WITH POLYCYSTIC DISEASE OF OTHER EPITHELIAL ORGANS

RECONSTRUCTION OF CYSTIC ELEMENTS IN TWO CASES *

ROBERT F. NORRIS, M.D.,† and RALPH M. TYSON, M.D.

(*From the Ayer Clinical Laboratory and the Pediatric Service of the Pennsylvania Hospital, Philadelphia, Pa.*)

Congenital polycystic disease of the lung is hard to define because the lesions are less uniform in structure than in polycystic disease of the kidney, liver, or pancreas and because in adults acquired lesions of the lungs may result in cystic dilatation of the bronchi or alveoli. Thus, there may be uncertainty whether the saccular dilatations of the bronchi seen in bronchiectasis are due primarily to infection acquired after birth or are due to a congenital defect aggravated by chronic secondary infection. There may also be uncertainty in some cases as to whether emphysematous dilatations of the alveoli in adults are acquired or congenital. The difficulty in classification is best illustrated by the list of terms applied to cystic lesions of the lung, compiled by Diamond and Durham,[1] such as pulmonary pneumocyst, bulbous emphysema, alveolar ectasia, cystic bronchiectasis, and open honeycomb lung. Recently a detailed classification of cystic disease of the lung, based on a review of the literature, has been made by Willis and Almeyda.[2,3] They classified the lesions into two large groups. The lesions of the first group are derived from the alveoli and include solitary alveolar cyst, or pneumatocele, and cystic emphysema. Those of the second group derived from bronchi include solitary bronchial cyst and multiple bronchial cysts, or cystic bronchiectasis. Although such a classification is valuable anatomically, it does not differentiate between lesions which are acquired or congenital and thus illustrates further how difficult it may be to decide in a given case whether cystic lesions are the result of a developmental abnormality or of disease beginning after birth. If equivocal cases of emphysema and bronchiectasis in adults are excluded from consideration, however, there is still a group of cystic lesions which are undoubtedly congenital. Thus, there is no dispute that solitary or multiple cysts of the lung manifest at birth or in infancy are the result of faulty development. From a review of the literature it is apparent that in most cases these cysts are derived primarily from bronchi and bronchioles and not from the alveoli.

When single or multiple cysts found in later life have no communications with the surrounding pulmonary tissue, the origin is usually considered congenital if in and about the lesions carbon pigment is not present. The lack of carbon particles is interpreted as meaning that from birth or infancy inspired air has not entered the cysts. The following discussion will be limited, therefore, to those cases of polycystic disease of the lung occurring in infancy which are definitely congenital.

In congenital polycystic disease of the lung, the lesions vary in size and extent. At times a large solitary cyst occupies most of one lobe and compresses the surrounding parenchyma. More often multiple cysts of varying size are scattered throughout one or more lobes of the lung. The cysts may be completely walled-off and filled with serous fluid or they may be air-containing as the result of small communications with functional bronchi. In addition, many patent bronchi associated with the lesions are irregularly dilated. Such lesions are often described as congenital bronchiectasis. Since the cysts are usually lined by epithelium, often ciliated, they are believed to be derived from bronchi, although in some cases the atria and alveolar ducts without epithelial lining are also described as cystic. When the latter occurs, it is usually spoken of as congenital emphysema. Some of the cysts apparently originate from the smaller bronchi and larger bronchioles, since the walls are partially invested with cartilage and nonstriated muscle and since mucous glands are present in the mucosa. Others are believed to arise from respiratory bronchioles and are composed entirely of a thin fibrous wall lined by flattened epithelium. In many of the smaller cysts and dilated but patent bronchioles, the columnar epithelium is reduplicated and projects into the lumina as papillary folds. Frequently some areas of the lung adjacent to the cysts are collapsed and the development of the alveoli may be less advanced than in the normal portions of the lung. Except in premature infants, however, many of the alveoli among the cysts are normally mature, lack a visible epithelial lining, and appear aerated.

When the lesions are extensive but the child survives after birth, infection of the dilated bronchi and cysts communicating with bronchi often causes fatal pneumonia and suppuration. This outcome is especially apt to occur when cystic lesions of the pancreas are present also. Recently the importance of these associated lesions has been emphasized in the pediatric literature (Andersen,[4,5] Wolman,[6] and Menten and Middleton [7]). It is reasoned that malnutrition resulting from deficiency in the external secretion of the pancreas makes the child liable to infection of the cystic lesions of the lung. When infection occurs, the manifestations at autopsy of acute or chronic inflammation are superimposed on the antecedent congenital lesions.

The fundamental cause of congenital polycystic disease in general is still unknown. As in other organs, the pulmonary lesions have been studied widely in order to determine the anatomic defect which initiates the changes resulting in single or multiple cysts. Yet there is no agreement on the manner in which the lesions are formed, probably because of uncertainty in many instances as to whether the lesions were actually congenital.

As long ago as 1880, Grawitz [8] thought that fetal bronchiectasis and cysts resulted from faulty anlagen and dilatation of lymph vessels. Stoerk [9] and Bernstein [10] believed that the irregular dilatation of the bronchi was secondary to stenosis caused by inflammation of the fetal lung or to excessive growth of the fetal mesenchyme surrounding the bronchi. Syphilis was suggested as a possible cause by Sandoz.[11] Buchmann,[12] Hueter,[13] and Scheidegger [14] believed that a tumor-like proliferation of the epithelium lining the bronchioles caused stenosis and saccular dilatations of the bronchi. In his extensive review of the literature up to that period, Koontz [15] concluded that stenosis of the bronchi or bronchioles is the essential lesion and that dilatation of the bronchioles and air spaces distal to the obstruction is secondary. King and Harris [16] suggested the possibility that local failure of canalization of the developing bronchioles associated with canalization distal to the lesions could account for isolation of cysts and stenosis with bronchiectasis of other bronchi. In a careful study of the cystic lung of a newborn premature infant, Wolman [17] concluded that buds from the trachea became pinched-off during embryologic development. Independent differentiation of these buds was thought to form a closed system of cysts.

Although careful studies of the polycystic lung have been published, little effort has been made to relate the findings with those in polycystic disease of other organs, such as the kidney, liver, and pancreas. In this connection, by means of serial sections and reconstructions, Norris and Herman [18,19] studied the lesions of polycystic kidney and we have studied those of polycystic liver [20] and pancreas.[21] In each organ the essential lesion appeared to be segmentation of the nephrons or of the biliary or pancreatic ducts, as the case may be, followed by isolation of the segments as cysts. Some of these cysts remain small, but others become widely dilated by cystic enlargement. There are indications that in many instances epithelial proliferation causing focal dilatation of tubules precedes the segmentation, but in others segmentation and isolation occur before cystic enlargement. Segmentation, however, does not occur until the epithelial unit is well differentiated and does not prevent the continued normal differentiation of the unit peripheral or distal to the lesions. This process is believed to be a degenerative

one and to be analagous with the normal resorption of the mesonephros, of the first generations of nephrons in the metanephros, and of many of the early intrahepatic bile ducts.

The present study was undertaken to determine whether the lesions occurring in the polycystic lung resemble those in the kidney, liver, and pancreas and whether the genesis of the lesions appears to be identical in all of the organs. Two infants were chosen for examination since the cystic lesions of the lung were unquestionably congenital and since, as previously emphasized, the pathogenesis of polycystic lesions can be more readily evaluated early in the course of the disease.

MATERIALS AND METHODS

Case 1 * (autopsy no. 3898) was previously reported in detail by Wolman.[17] Briefly, the child was a stillborn Negro infant weighing 915 gm. and measuring 30 cm. in length. The age was considered to be 6 lunar months. Case 2 (autopsy no. 6489) was a full-term white infant, weighing 2550 gm. and measuring 49 cm. in length, who died 4½ hours after delivery of clinically unexplained respiratory failure. In both cases, the significant lesions found at necropsy were confined to the lungs and the remaining organs will not be described.

At autopsy all tissues were fixed either in Regaud's or Kaiserling's solutions. Blocks were embedded in paraffin and stained with either Delafield's or Harris' hematoxylin and eosin. In addition to ordinary sections cut at 5 μ, in each case several hundred serial sections, 15 μ in thickness, were cut from two cubical blocks about 1.5 cm. on a side. Cystic lesions and distorted bronchi and bronchioles were studied and traced through these sections. Some of the lesions were reconstructed by the method previously described.[18,19]

Case 1

Gross Examination. The right lung occupied most of the thoracic cavity. The heart was displaced to the left, laterally and dorsally, and the left lung was small and compressed. Except for atelectasis the left lung was also microscopically normal. The enlargement of the right lung was caused by diffusely scattered, smooth-lined cysts, varying in size from 1 mm. to 1 cm. in diameter. These have been previously illustrated.[17] Although the cysts were completely isolated from the main bronchi, small circular communications were demonstrated between many of them. The cysts were filled with colorless watery fluid which could not be expressed on pressure. In the dorsal part of the lung was a zone of dense parenchyma, resembling the collapsed left

---

* Dr. Wolman has kindly consented to our use of his case in the present study.

lung, in which no cysts were seen. The right bronchus and its three main branches were patent but the latter ended abruptly and blindly in the cystic tissue. Smaller bronchi could not be traced.

Microscopic Examination. The large cysts were composed entirely of thin layers of fibrous stroma lined by a single layer of flattened epithelium. In some the epithelial lining was lacking. The walls of the smaller cysts were thin and fibrous and these were lined by a single layer of columnar epithelium, often ciliated. Only in areas near the hilum was smooth muscle visible in the walls. The muscle fibers were thin and irregular and usually did not completely encircle the cysts. Also there were but a few small atypical islands of cartilage which likewise did not encompass the cysts. Most of the smaller cysts were irregularly oval or circular. In serial sections, all of the cysts which were traced were found to be completely isolated from the rest of the bronchial tree, but anastomoses between cysts were common. In addition, many outpocketings and relatively undilated branches projected laterally from the cysts. Instead of rebranching into numerous subdivisions in continuity with the ramifications of the air spaces, these branches ended abruptly. Along their courses, however, were many small atypical channels of communication with the terminal air sacs (Fig. 1). Among the cysts were a few, small, undilated bronchi or bronchioles which also were lined by columnar epithelium and which, when traced, were found to end blindly at the proximal ends. These likewise communicated with the air sacs by irregularly spaced slit-like apertures and distally often ended bluntly without subdivision. The terminal air sacs were nearly circular and were expanded. Some contained freshly extravasated erythrocytes. The air sacs were lined by single layers of cuboidal epithelium which was not reduplicated and which did not form papillary projections. The cytoplasm of the cells was more translucent than that of the epithelium which lined the cysts and undilated bronchi. The stroma about the cysts and air sacs was composed of relatively loose connective tissue in which elastic fibers could not be demonstrated with Verhoeff's stain. The number of capillaries appeared normal and many of them were pushing through the walls of the air sacs and the lining epithelium to lie exposed in the air spaces. Nowhere was there evidence of inflammatory exudate (Fig. 2). Two of the cysts have been reconstructed to show the globular contours, the bluntly-ending outpocketings, and the occasional anastomoses (Fig. 5).

In the spongy part of the lung, previously referred to, there were no cysts. This area was surrounded and encroached upon by the cystic portions of the lung. A central core was made up of compact fibrous

tissue in which large blood vessels divided and fanned out toward the periphery. Encircling this core was a mass of collapsed air sacs which also were lined by cuboidal epithelium. Highly irregular but collapsed bronchioles were scattered throughout the parenchyma (Fig. 3). The lining epithelium was columnar and ciliated. There was no investment with smooth muscle or cartilage. Many bronchioles were traced in serial sections and all terminated blindly in at least one direction, but all of them ended distally in collapsed alveolar ducts or atria. Anastomoses between the proximal ends were numerous (Fig. 4). To illustrate the irregularity and patternless arrangement of the bronchioles, several have been reconstructed (Fig. 6).

Case 2

Gross Examination. The great vessels and heart, which lay entirely in the right thorax, were completely transposed. The right lung, although collapsed, was otherwise normal grossly and microscopically. The upper lobe of the left lung was entirely replaced by a large globular cyst filled with serous fluid. The lining was smooth but the wall was irregularly thickened with fibrous trabeculae. The bronchus of the upper lobe was small and ended blindly in the wall of the cyst. In the lower lobe of the left lung the large bronchi were normal and unobstructed. This was the only lobe of either lung which was expanded and air-containing. No gross lesions were seen.

Microscopic Examination. The wall of the large cyst in the upper lobe of the left lung was composed entirely of irregularly thickened fibrous tissue in which small blood vessels were still visible. A single layer of flattened epithelium lined the cyst. There was no evidence of inflammation.

In the lower lobe of the left lung, which appeared normal grossly, the larger bronchi were not remarkable. The medium-sized bronchi were slightly irregular and the cartilages were not symmetrically spaced. Trabeculae of loose fibrous tissue partially surrounded a few of these bronchi (Fig. 7). Many but not all of the distal bronchi and bronchioles, however, were distinctly abnormal. These bronchi were atypically spaced and small saccular dilatations were numerous. Cartilages were absent, but strands of muscularis usually completely encircled the bronchi beneath the mucosa, and also extended laterally to anastomose with the muscular layers of other bronchi. The epithelial lining was uniformly columnar and ciliated and was often thrown into regular folds as if by contraction of the muscularis. When traced in serial sections, none of the bronchi and bronchioles were obstructed or interrupted. Distally the bronchioles repeatedly subdivided and com-

municated with the complex of air spaces by narrow slit-like apertures. On the other hand, there were numerous short lateral branches which did not subdivide normally, but which also communicated with the air sacs by narrow channels. The alveoli in this lobe were diffusely expanded and many of them contained erythrocytes and necrotic squamous epithelial cells. In most areas an epithelial lining was not visible. When present, the lining was composed of a single layer of flattened epithelial cells which usually was detached from the alveolar wall. Fetal mesenchyme was almost completely lacking among the alveoli and the alveolar capillaries were supported only by elastic tissue and a delicate reticulum. There was no evidence of inflammation (Fig. 8). The saccular dilatations and irregularity in contour of the smaller bronchi are illustrated in the reconstruction (Fig. 9).

DISCUSSION

The two cases differ in certain details. In case 1 diffusely occurring cysts, apparently of bronchial origin, were completely isolated from the main bronchial tree. Scattered among the cysts were undilated bronchioles, the proximal ends of which also did not communicate with the main bronchi. In the dorsal segment of the lung collapsed bronchioles and air sacs which showed no evidence of cystic dilatation also were completely isolated. By contrast, in case 2 a solitary large cyst completely replaced the upper lobe of the left lung. Many of the bronchioles in the lower lobe of this lung, however, were highly irregular in contour as the result of numerous saccular dilatations, but so far as could be determined were not broken up into isolated segments.

In both lungs, however, the terminal air sacs or alveoli were remarkably normal in appearance and were mature for the age of the fetus. Although atypically situated, numerous open communications existed in both cases between the bronchioles and the terminal air sacs. Likewise in both cases, the mesenchyme was normally mature for the age of the fetus and evidence of inflammation was lacking. Except for the upper lobe of the left lung in case 2 in which no alveoli were seen, the air sacs about the cystic lesions were expanded in both cases. The immature alveoli in the cystic portion of the lung in case 1 appeared to be fully expanded, but were collapsed in the noncystic dorsal area. This expansion was thought to be slightly in excess of normal physiologic canalization. For an explanation of this phenomenon, it will be recalled that both the larger and smaller cysts intercommunicated and that the smaller cysts and undilated bronchioles had numerous branches interpreted as atria, which communicated with the alveolar complex. Since the larger cysts were filled with fluid, it is quite

likely that, as the result of communicating channels, the air sacs were likewise distended with fluid. The lack of elastic tissue in the enveloping mesenchyme also would favor expansion of the alveoli under these circumstances. In the dorsal part of the lung, in which there were no cysts and in which no fluid presumably, had accumulated, compression by the surrounding cysts resulted in collapse and arrested development of the bronchioles and air sacs. A different situation existed in the lower lobe of the left lung in case 2. In spite of the saccular dilatation of the bronchi in this lobe, there was no apparent obstruction of the bronchial tree and many of the alveoli contained aspirated squamous epithelial cells. The expansion of the alveoli was therefore physiologic.

Although the two cases thus show both similarities and differences, it is thought that the underlying defect is the same. If this supposition is true, the slight irregular dilatation and distortion of the bronchioles among the cysts of the right lung in case 1 and in the lower lobe of the left lung in case 2 may be considered the first stage in the development of polycystic disease. The appearance of the lesions suggests that the focal dilatations were caused by expansion of the single layers of epithelial cells which lined the bronchi. However, it has been previously emphasized that segmentation may at times antedate dilatation of the isolated segments.[18-21] In this connection, furthermore, it should be emphasized also that segmentation of the bronchioles is believed to occur only after differentiation of the epithelium. This assumption is supported by the fact that the epithelium lining the dilated segments and cysts was columnar and often ciliated. If the progress of the disease is arrested in the stage of focal dilatation without segmentation, the lesions may be called fetal bronchiectasis. Such lesions may well be the origin of some cases of bronchiectasis in later life. When the lesions progress beyond the stage of focal dilatation, slightly dilated segments of bronchi or bronchioles become pinched off to form the gradually enlarging cysts found in the right lung of case 1 or the large solitary cyst in the upper lobe of the left lung in case 2. Even though partially or wholly disconnected from the main bronchial tree, the air sacs and alveoli distal to the bronchial lesions continue to differentiate in a relatively normal manner.

This course of events is compatible with the steps in the normal development of the lung according to Miller,[22] Norris, Kochenderfer, and Tyson,[23] and Patten.[24] The lungs originate from a ventral laryngotracheal outgrowth of entoderm which divides into two bronchial buds at about 4 mm. At the beginning of the second month, or at about 8.5

mm., the main bronchi are established. After that, the ramifications of the bronchial tree and the terminal air sacs are formed by successive divisions, either dichotomous or monopodial, until differentiation is almost complete at the time of birth. During this period the terminal air sacs are lined by continuous layers of epithelium until shortly before birth so that differentiation and subdivision of the terminal elements of the bronchial tree are always concerned with epithelial structures. Since it has been emphasized previously that segmentation of an epithelial structure is a degenerative or resorptive process, it is altogether likely that focal dilatation and isolation of segments of bronchi in the present cases occurred only after normal proliferation and subdivision was proceeding distal to the lesions and was not seriously impaired by the lesions.

In the kidney (Kampmeier *et al.*[25-30]) and liver (Lewis [31]) it is known that the early generations of nephrons and intrahepatic bile ducts, respectively, are normally resorbed. In the lung, however, it has not been established that any bronchi and bronchioles are normally provisional. Norris, Kochenderfer, and Tyson,[23] however, in a study of the normal lung found evidence in a 15 cm. fetus which suggested to them that some elements of the developing lung may regress and be resorbed, perhaps as the result of the asymmetry of the relatively fixed thoracic cage. If this observation is correct, precedent for the type of degeneration described in the polycystic lung may be present in the development of the normal lung.

For the polycystic kidney, Teuscher [32] suggested that an over-production of excretory tubules, of which some become isolated as cysts, might explain the origin of polycystic disease. Such a theory would be supported by the frequent association of polydactylism with polycystic lesions of the internal organs. The appearance of an excessive number of epithelial elements contributed to the belief of Brigidi and Severi,[33] Nauwerck and Hufschmid,[34] and Staemmler [35] that polycystic disease is a true fetal neoplasm or cystadenoma. Although Norris and Herman [19] could not demonstrate an excessive number of renal tubules in the kidneys which they studied, we [20] did find that the number of intrahepatic bile ducts in a case of polycystic liver appeared to be greater than normal. However, this phenomenon might well be due to the inherent tendency of bile ducts to regenerate following functional and anatomic disturbances. In the right lung of case 1, the number of bronchial elements also appears to be excessive. This appearance (Fig. 1) may be due to the isolation of bronchial segments which, when expanded, tend to occupy more of the parenchyma than is normal. Since

the alveoli continue to differentiate in the presence of bronchial obstruc-
tion *it is also possible that isolated segments of bronchi may continue to*
differentiate and branch after their separation from the main bronchial
tree. Such a phenomenon would account for the apparently increased
number of bronchial elements and the anastomoses between cysts and
bronchi (Figs. 5 and 6). It might also explain why isolated segments
of bronchi remain as gradually enlarging cysts instead of disappearing.
Furthermore, when sections of the lower lobe of the left lung in case 2,
in which there were no isolated bronchial segments, were surveyed, the
number of bronchi and bronchioles did not appear to be excessive.
Consequently, there is as yet no definite evidence that an excessive
formation of epithelial elements precedes the lesions of polycystic
disease.

In regard to the large solitary cyst in the left lung of case 2, Hep-
ler [36] showed experimentally that in the rabbit kidney obstruction of
the papilla did not result in cystic enlargement of the organ. When the
arterial supply and the papilla were both obstructed, however, large
solitary cysts of the kidney were apt to occur. In the present case it
will be recalled that the heart and great vessels were completely trans-
posed. Although the arterial supply of the right lung was not ade-
quately studied at autopsy, it is quite possible that a circulatory
anomaly may have been responsible for the large cyst in the upper
lobe. Furthermore, it may be said that in general the etiologic signifi-
cance of deficiencies or abnormalities of circulation in polycystic dis-
ease has not been studied widely.

Since the lesions of polycystic lungs are therefore similar to those
which have been described in other epithelial organs, the sequence of
anatomic changes proposed for the lungs is identical with that previ-
ously suggested for the kidney, liver, and pancreas. In all of these
organs, the functional elements are generally distal to their points of
attachment to the rest of the body and are connected with the body by
ducts or stalks which function only as communicating channels. In
general, the lesions of polycystic disease, consisting in segmentation
either with or without focal dilatation, originate in these ducts. In the
normally provisional mesonephros, likewise, physiologic resorption of
the organ begins by segmentation of the collecting ducts before degen-
eration of the glomeruli is initiated (Felix [37]). Thus by analogy it ap-
pears that in the polycystic organ the segmentation of the ducts is also
a manifestation of resorption or degeneration. It is concluded, there-
fore, that in polycystic disease the affected organ is more or less ab-
normally provisional as the result of an unexplained defect in fetal
development.

SUMMARY

The polycystic lesions in the lungs of two infants were studied and reconstructions of the cystic elements were made from serial sections.

The fundamental lesion appears to be focal segmentation preceded or followed by focal dilatation of the small bronchi and bronchioles.

If the disease becomes arrested in the stage of focal dilatation without segmentation, bronchiectasis results which may persist for the duration of life.

When the bronchi are broken up into isolated segments, some of these segments persist as gradually enlarging cysts. The well known lesions of polycystic disease are thus established.

The lesions and sequence of anatomic changes in the polycystic lung are similar to those previously described in the polycystic kidney, liver, and pancreas.

In general, the lesions of polycystic epithelial organs primarily affect the nonfunctioning system of ducts.

By analogy with the process of physiologic resorption in normally provisional organs or parts of organs, it is concluded that polycystic disease is a pathologic manifestation of normal fetal resorption and degeneration. The polycystic organ is therefore partially provisional.

The fundamental defect which initiates the developmental anomalies is unknown and the etiologic importance of anomalies of circulation in polycystic organs has not been determined.

REFERENCES

1. Diamond, S., and Durham, W. R. Cystic lungs. *Am. Rev. Tuberc.*, 1940, 41, 719–731.
2. Willis, F. E. S., and Almeyda, J. Cystic disease of the lung (broncho-alveolar cysts). Part I. *Tubercle*, 1943, 24, 27–36.
3. Willis, F. E. S., and Almeyda, J. Cystic disease of the lung (broncho-alveolar cysts). Part II. *Tubercle*, 1943, 24, 43–58.
4. Andersen, D. H. Cystic fibrosis of the pancreas and its relation to celiac disease. A clinical and pathologic study. *Am. J. Dis. Child.*, 1938, 56, 344–399.
5. Andersen, D. H. Cystic fibrosis of the pancreas, vitamin A deficiency, and bronchiectasis. *J. Pediat.*, 1939, 15, 763–771.
6. Wolman, I. J. Cystic fibrosis of the pancreas. *Am. J. M. Sc.*, 1942, 203, 900–906.
7. Menten, M. L., and Middleton, T. O. Cystic fibrosis of the pancreas. Report of 18 proved cases. *Am. J. Dis. Child.*, 1944, 67, 355–359.
8. Grawitz, P. Ueber angeborene Bronchiektasie. *Virchows Arch. f. path. Anat.*, 1880, 82, 217–237.
9. Stoerk, O. Ueber angeborene blasige Missbildung der Lunge. *Wien. klin. Wchnschr.*, 1897, 10, 25–31.
10. Bernstein, J. M. Broncho-pneumonia with multiple cavities. *Tr. Path. Soc. London*, 1905, 56, 330–333.

11. Sandoz, E. Über zwei Fälle von "fötaler Bronchiektasie." *Beitr. z. path. Anat. u. z. allg. Path.*, 1907, 41, 495–516.

12. Buchmann, E. Zur Lehre der fötalen Lungenatelektase und der fötalen Bronchiektase. *Frankfurt. Ztschr. f. Path.*, 1911, 8, 263–303.

13. Hueter, C. Über angeborene Bronchiektasien und angeborene Wabenlunge. *Beitr. z. path. Anat. u. z. allg. Path.*, 1914, 59, 520–538.

14. Scheidegger, S. Kongenitale Bronchiektasien. *Frankfurt. Ztschr. f. Path.*, 1934, 47, 276–284.

15. Koontz, A. R. Congenital cysts of the lung. *Bull. Johns Hopkins Hosp.*, 1925, 37, 340–361.

16. King, J. C., and Harris, L. C., Jr. Congenital lung cyst. *J. A. M. A.*, 1937, 108, 274–280.

17. Wolman, I. J. A case of congenital cystic lung in a stillborn fetus. *Bull. Ayer Clin. Lab., Pennsylvania Hosp.*, 1930, no. 12, 49–54.

18. Norris, R. F., and Herman, L. Observations on the anatomic basis of congenital polycystic kidneys. *Tr. Am. A. Genito-Urin. Surgeons*, 1938, 31, 41–57.

19. Norris, R. F., and Herman, L. The pathogenesis of polycystic kidneys: reconstruction of cystic elements in four cases. *J. Urol.*, 1941, 46, 147–176.

20. Norris, R. F., and Tyson, R. M. The pathogenesis of polycystic livers. Reconstructions of cystic elements in two cases. *Am. J. Path.*, 1947, 23, 201–215.

21. Norris, R. F., and Tyson, R. M. The pathogenesis of polycystic pancreas. Reconstruction of cystic elements in one case. *Am. J. Path.*, 1947, 23, 485–499.

22. Miller, W. S. The Lung. Bailliére, Tindall, and Cox, London, 1937.

23. Norris, R. F., Kochenderfer, T. T., and Tyson, R. M. Development of the fetal lung, with special reference to the lining of the alveoli and the effect of immaturity on respiration. *Am. J. Dis. Child.*, 1941, 61, 933–950.

24. Patten, B. M. Human Embryology. The Blakiston Co., Philadelphia, 1946, 776 pp.

25. Kampmeier, O. F. Über das Schicksal der erstgeformten Harnkanälchen der bleibenden Niere beim Menschen. *Arch. f. Anat. u. Physiol., anat. Abteil.*, 1919, 204–226.

26. Kampmeier, O. F. A hitherto unrecognized mode of origin of congenital renal cysts. *Surg., Gynec. & Obst.*, 1923, 36, 208–216.

27. Kampmeier, O. F. Weitere Studien über die Entwicklungsgeschichte der bleibenden Niere beim Menschen. *Ztschr. f. Anat. u. Entwcklngsgesch.*, 1924, 73, 459–500.

28. Kampmeier, O. F. The metanephros or so-called permanent kidney in part provisional and vestigial. *Anat. Rec.*, 1926, 33, 115–120.

29. McKenna, C. M., and Kampmeier, O. F. A consideration of the development of polycystic kidney. *Tr. Am. A. Genito-Urin. Surgeons*, 1933, 26, 377–383.

30. McKenna, C. M., and Kampmeier, O. F. A consideration of the development of polycystic kidney. *J. Urol.*, 1934, 32, 37–43.

31. Lewis, F. T. The Development of the Liver. In: Keibel, F., and Mall, F. P. (eds.) Manual of Human Embryology. J. B. Lippincott Co., Philadelphia, 1912, 2, 403–428.

32. Teuscher, M. Über die kongenitale Cystenleber mit Cystennieren und Cystenpankreas. *Beitr. z. path. Anat. u. z. allg. Path.*, 1926, 75, 459–485.

33. Brigidi, V., and Severi, A. Contributo alla patogenesi delle cisti renali. *Sperimentale, Arch. di biol.*, 1880, 46, 1–25.

34. Nauwerck, C., and Hufschmid, K. Ueber das multiloculäre Adenokystom der Niere. *Beitr. z. path. Anat. u. z. allg. Path.*, 1893, 12, 1–28.

35. Staemmler, M. Ein Beitrag zur Lehre von der Cystenniere. *Beitr. z. path. Anat. u. z. allg. Path.*, 1921, 68, 22–57.

36. Hepler, A. B. Solitary cysts of the kidney. A report of seven cases and observations on the pathogenesis of these cysts. *Surg., Gynec. & Obst.*, 1930, 50, 668–687.

37. Felix, W. The Development of the Urogenital Organs. In: Keibel, F., and Mall, F. P. (eds.) Manual of Human Embryology. J. B. Lippincott Co., Philadelphia, 1912, 2, 752–979.

[Illustrations follow]

DESCRIPTION OF PLATES

PLATE 157

FIG. 1. Case 1. Cystic portion of right lung. Photomicrograph shows small, irregular cysts lined by single layers of ciliated columnar epithelium. Among the cysts the air sacs are uniformly expanded. To the right of center are three undilated bronchioles and a small artery. × 45.

FIG. 2. Case 1. Cystic portion of right lung. Photomicrograph shows the character of the darkly staining columnar epithelium of the bronchioles in contrast with the translucent cuboidal epithelium which lines the air sacs. In the lower left corner, the slit-like breaks in the bronchial epithelium are atypical communications with the air sacs. × 185.

1

2

FIG. 3. Case 1. Atelectatic portion of the right lung. Photomicrograph shows a fibrous central core in which large blood vessels branch toward the periphery. The bronchioles are small and irregular. In the lower left center there is an anastomosis between two bronchioles. In the lower left corner, the cystic portion of the lung is visible. × 45.

FIG. 4. Case 1. Atelectatic portion of right lung. Photomicrograph shows that the air sacs lined by single layers of translucent cuboidal epithelial cells are collapsed. Communications between the two bronchioles in the center and the air sacs are seen at the points where the darkly stained epithelium ends abruptly. × 185.

Norris and Tyson

Congenital Polycystic Lung

PLATE 159

FIG. 5. Case 1. Cystic portion of right lung. Reconstruction shows the dilatation and anastomosis between two small cysts. Several atypical undilated branches and outpocketings which show varying degrees of distortion are illustrated. Not shown are the communications between these branches and the air sacs. The approximate vertical extent of the reconstruction in the lung was 4.53 mm.

FIG. 6. Case 1. Atelectatic portion of right lung. Reconstruction shows the irregularity of the undilated bronchioles, the atypical distribution, and the anastomoses between them. The shaded background represents collapsed air sacs. Not shown are the communications between bronchioles and air sacs. The approximate vertical extent of the reconstruction in the lung was 0.98 mm.

PLATE 159

Congenital Polycystic Lung

5

11

PLATE 160

FIG. 7. Case 2. Lower lobe of left lung. Photomicrograph shows that a medium-sized bronchus to the left of center is surrounded by atypically spaced cartilages. Mucous glands extend outward between the cartilages. To the right of center are two bronchioles. Narrow openings between the bronchioles and expanded alveoli are apparent. × 45.

FIG. 8. Case 2. Lower lobe of left lung. A high power photomicrograph of a bronchiole seen in Figure 7 shows clearly the communications between the bronchiole and the alveoli and the regularity of the mucosal folds. The interlacing bands of nonstriated muscle between bronchioles are shown also. Some alveoli are devoid of visible epithelium; others are incompletely lined by single layers of cuboidal epithelium. × 185.

PLATE 160

AMERICAN JOURNAL OF PATHOLOGY. VOL. XXIII

7

8

PLATE 161

FIG. 9. Case 2. Lower lobe of left lung. Réconstruction shows the irregularity and cystic dilatations of the small bronchi. Most of the branches are blunt and end abruptly without the usual subdivisions. A few of the branches are slender and undilated. Not shown are the narrow channels of communication between the bronchioles and the alveoli. The approximate vertical extent of the reconstruction in the lung was 1.22 mm.

PLATE 161

Congenital Polycystic Lung

Norris and Tyson

GINGIVAL BIOPSY FOR THE DIAGNOSIS OF GENERALIZED AMYLOIDOSIS*

Irving J. Selikoff, M.D., and Edward H. Robitzek, M.D.

(From the Medical Service of Sea View Hospital, Staten Island, N.Y.)

Generalized amyloidosis is a frequent complication of many chronic diseases, particularly tuberculosis. Its reported incidence varies with the thoroughness with which it is sought, but in several large series of post-mortem studies it has been shown to occur in approximately 20 per cent of patients with tuberculosis who are autopsied.[1-3] Its clinical incidence is lower than this, probably averaging about 10 per cent of patients with "moderately" to "far advanced" pulmonary tuberculosis. In these patients, the presence of generalized amyloidosis is an important complication in the management of the disease, depriving some of the opportunity for life-saving surgery, and in others being the direct cause of death, especially if massive hepatic, adrenal and, particularly, renal amyloid deposits are present.

For the clinician, the recognition of the onset and the presence of amyloidosis is often difficult, unless the characteristic triad of hepatosplenomegaly, proteinuria, and edema are present. However, this syndrome is usually absent, having been noted, in one careful study, in less than 25 per cent of cases of proved amyloidosis.[4] In the remaining cases, as well as those in which the clinical diagnosis is suspected, the Congo red test is of considerable diagnostic value. Introduced by Bennhold[5] in 1923, this test may detect the presence of amyloidosis even in the absence of clinical findings. It is based upon the "absorption" of the injected colloidal dye by amyloid deposits in the tissues so that upon examination of the circulating blood 1 hour after the injection little or no dye is found remaining. However, this test is deficient in several respects. Frequently, a "positive" test will occur in patients who have no amyloidosis[6] and although definitive criteria can be established to avoid this error,[7] this involves repeated testing, a procedure not altogether innocuous.[8] Secondly, and perhaps more important, there is the inability of the test to demonstrate moderate or small amounts of amyloid; in these cases only limited amounts of Congo red will be removed from the blood stream and much will still be present when a sample is examined 1 hour after injection. But partial absorption is also found in patients without amyloidosis, so that the Congo red test, when it shows incomplete absorption, is without differential diagnostic value.[9] To the present, there is no satisfactory diagnostic method for the determination of amyloidosis in patients with but moderate amounts of amyloid.

Tissue biopsy for the diagnosis of amyloidosis was used before the introduction of the Congo red test, being employed for this purpose by Josefson [10] (spleen) and Waldenström [11] (liver). Biopsy of the liver has been performed since, but only infrequently since aspiration is not without danger, while laparotomy is not readily acceptable to the patient. Tissue biopsy has also been extensively used in experimental animal studies on amyloidosis, enabling several investigators to fix the time of onset of the amyloid and provide proof of its regression.[12-14] In the uncommon, primary ("atypical") systematized amyloidosis, biopsy has also been done of other tissues, such as skin,[15-17] stomach,[18] muscle,[16,19] tendon,[20] mucous membranes,[21] and tongue.[19,21-23] In these patients, however, gross abnormalities were present and biopsy was done to study the nature of that abnormality: in most cases, the discovery of atypical amyloid disease came as a surprise. The situation in generalized amyloidosis is not comparable. There are no easily available tissues which appear grossly abnormal; certainly, no superficial tissues. The normal appearance of the skin and mucous membranes in generalized amyloidosis gives no indication that they might contain an unusual substance.

Despite this normal appearance, however, there is good reason to suspect that these tissues, particularly the gingivae, might contain amyloid. Amyloid substance, when present, is widely distributed, and there are no grounds on which to expect that it would be absent from the gingivae. Moreover, since perivascular areas are sites of predilection for the deposition of amyloid, the vascular nature of the gingivae makes them a fertile site in which to seek this material. This has been emphasized previously.[1] Technically, biopsy in this region is simple and not forbidding to the patient, can be repeated, and carries no danger. Finally, the anatomic structure of the gingivae is peculiarly suitable to the procedure since the vessels are not only numerous, but are also surrounded by intertwining bundles of fibrous tissue which makes excessive bleeding uncommon. Few pain nerve endings are present [24] and the tissue is also markedly resistant to infection, again making it suitable for the purpose, particularly since it is so accessible.

MATERIAL AND METHOD

In an effort to ascertain whether biopsy of oral tissues would be of value in the diagnosis of generalized amyloidosis, a study was recently undertaken at Sea View Hospital in which a number of such examinations were done.

Fifty patients, each suffering from pulmonary or osseous tuberculosis, were chosen for study, but in 3, insufficient material was obtained

for microscopic examination. Therefore, for the purposes of this investigation, the remaining 47 patients will be considered consecutive.

The patients were in three groups. In the first were 18 patients in whom the diagnosis of generalized amyloidosis had been made with considerable confidence. The second group contained 21 patients in whom this diagnosis had been ruled out, so far as was clinically possible. The third group was composed of 8 patients in whom the diagnosis was being considered but could not be made definitely. Every patient was thoroughly studied clinically, complete physical examinations were performed, and laboratory studies done. Urine examinations included multiple tests for proteinuria, casts, red blood cells, specific gravity and, in many instances, protein fractionation for albumin and globulin. Biochemical studies included those for plasma proteins, non-protein nitrogen, and for hepatic and renal function. Each patient was tested with the Congo red test, in many cases several times. The technic used here was Taran's modification[25] of the Bennhold method, which avoids the inaccuracies caused by colorimetric difficulties in the presence of hemolysis, and which has been accepted as standard by the Committee on Standard Laboratory Procedure of the American Trudeau Society.[26]

The specimens were taken in the Dental Department and consisted of pieces of tissue measuring approximately 3 by 5 mm. Two sites were used, the gingiva proper and the mucobuccal fold, since no information was available as to the suitability of either. Upon sections from each specimen three stains were used: hematoxylin and eosin, Congo red, and methyl violet. It has been the experience of the pathologic laboratories of this institution that methyl violet staining is most satisfactory, and this has been the finding in this study as well. Its only drawback is the difficulty of making permanent preparations. We have found that methyl violet was much more specifically limited to the amyloid material, and gave greater contrast with the non-amyloid tissues, than did Congo red. In the liver, for example, the latter stains the perivascular fibrous tissues a pink-brown shade not unlike the truly pink amyloid; the same difficulty is found in the vicinity of Glisson's capsule. In the gingiva, moreover, the fibrous, hyaline, and muscular elements in the subepithelial layers are also widely stained an off-pink hue by the Congo red, as is coagulated serum, so that differentiation from the amyloid deposits is difficult. When guided by comparison with a section stained with methyl violet, amyloid can be differentiated by Congo red, but when Congo red is used by itself, it is apt to be confusing. With both stains, too, diffuse daylight is preferable. Hematoxylin and eosin we found of little value.

Anatomic correlation was attempted by biopsy of other tissues in several cases, and was obtained at autopsy in others.

RESULTS

The results of our study indicate that biopsy of the oral mucosa, especially the gingiva, can be of considerable value in the diagnosis of generalized amyloidosis.

Our findings in the first group (patients with amyloidosis) are summarized in Table I. Here, in 14 of the 18 patients studied, amyloid deposits were found in the tissues examined. It should be emphasized that these tissues, as in all patients investigated, were grossly normal. These results are more significant when it is realized that the complete picture of hepatosplenomegaly, edema, and proteinuria was present in only 2 of these cases, yet amyloid was demonstrated by biopsy in 13 of the remaining 16. This and other correlations are summarized in Table II. They emphasize that the absence of any one sign or group of signs does not exclude the possibility of finding amyloid by gingival biopsy. In case 14, for example, only proteinuria was present and there was no palpable liver or spleen and no edema. Yet gingival biopsy revealed amyloid. The converse is also true. Our data show that the presence of any one finding or combination of findings is no guarantee that amyloid will be present in a specimen taken for biopsy.

Failure to find amyloid by gingival biopsy does not rule out the disease. In 2 of the 4 cases with no amyloid in the material submitted for biopsy, we had the opportunity of examining other tissues. In both, amyloid was found, leading to the impression that clinical diagnosis, based upon repeated demonstration of complete or nearly complete Congo red absorption,[7] may be correct in the face of negative gingival biopsy. Thus, in case 5, the patient gave every evidence of generalized amyloidosis, including hepatosplenomegaly, edema, marked proteinuria, and repeated complete absorption of injected Congo red, yet careful search of the oral specimen failed to reveal amyloid deposits. One week later, biopsy of the liver showed massive amyloid infiltration. Even more instructive was case 7, which repeatedly showed complete absorption of Congo red, although proteinuria was the only significant finding, edema and enlargement of the liver or spleen being absent. Biopsy in this case, too, was negative. Nevertheless, autopsy 3 weeks later revealed amyloidosis of the liver, spleen, adrenals, and kidneys, with death due to nephropathia amyloidea. It seems obvious that negative biopsy does not rule out generalized amyloidosis. A positive biopsy, on the other hand, is valuable, particularly in circumstances outlined below.

TABLE I

Oral Biopsy in Patients with Clinically Diagnosed Generalized Amyloidosis

Case	Clinical examination			Laboratory studies			Clinical diagnosis	Oral biopsy			Examination of other tissues
	Enlargement		Edema	Proteinuria	Congo red absorption	Chemical examination of blood		Site	Result	Extent	
	Liver	Spleen									
1.	++++*	o	+	++++	100, 100, 100	Hypoproteinemia	Amyloidosis	Mucobuccal fold	Positive	+++	Necropsy: generalized amyloidosis
2.	++++	+	++++	+++	100, 100	Hypoproteinemia	Amyloidosis	Mucobuccal fold	Positive	+	
3.	+++	o	o	++	100, 100, 100	Minimal azotemia	Amyloidosis	Gingiva	Positive	+++	
4.	o	++	o	+	100, 100, 100	Normal	Amyloidosis	Mucobuccal fold	Negative		Liver biopsy: amyloidosis, +++
5.	+++	+	+++	++++	100, 100	Hypoproteinemia	Amyloidosis	Gingiva	Negative		Necropsy: generalized amyloidosis
6.	++	o	+++	++++	97, 85, 100	Hypoproteinemia, marked azotemia	Amyloidosis	Gingiva	Positive	++	
7.	o	o	o	++++	100, 100, 100	Marked azotemia	Amyloidosis	Mucobuccal fold	Negative		Necropsy: generalized amyloidosis
8.	+++	o	o	++++	100, 100, 100	Minimal azotemia	Amyloidosis	Gingiva	Positive	++	
9.	+++	+	o	++	100, 100, 100	Normal	Amyloidosis	Mucobuccal fold	Positive	+	
10.	++	+++	o	++++	100, 100, 100	Moderate azotemia	Amyloidosis	Mucobuccal fold	Positive	+++	Prostatic resection; specimen, amyloid ++
11.	o	++	o	++	100, 100, 100	Normal	Amyloidosis	Mucobuccal fold	Positive	+	Nephrectomy for renal tuberculosis; specimen, amyloid ++
12.	+++	o	o	++++	100, 100, 100	Minimal hypoproteinemia	Amyloidosis	Mucobuccal fold	Positive	+++	
13.	+	o	o	++++	98, 90, 100, 100	Normal	Amyloidosis	Mucobuccal fold	Positive	+	
14.	o	o	o	++++	100, 100, 100	Azotemia	Amyloidosis	Gingiva	Positive	+	Necropsy: generalized amyloidosis
15.	++	o	o	++++	100, 95, 100	Azotemia	Amyloidosis	Mucobuccal fold	Positive	+	
16.	++++	++++	o	+	100, 100, 100	Normal	Amyloidosis	Gingiva	Positive	+	
17.	+	o	o	+	100, 100	Normal	Amyloidosis	Gingiva	Positive	++	
18.	o	o	o	+	98, 98, 100	Normal	Amyloidosis	Gingiva	Negative		

* Gradings in pluses are arbitrary. A one-plus is definite, albeit minimal; a four-plus indicates massive amounts.

There was no constant correlation between the extent of the amyloid deposits found in the gingivae and the severity of the amyloidosis. In the 2 patients with every sign of amyloidosis, one, as noted, showed no amyloid in the gingival tissue while the other had it in but minimal amounts. There was, in particular, no correlation between the amount in the gingivae and that in the kidney: 4 patients in this group have been autopsied since the biopsy and in the one patient (case 1) who showed rather large amounts in the gingivae, only moderate infiltration of the kidneys was present. The other 3 showed little or no amyloid in the gingivae (cases 6, 7, and 14) yet each had massive amyloido-

TABLE II

Correlations between Findings in Oral Specimens for Biopsy and Absence of Evidence of Visceral Involvement in Generalized Amyloidosis (18 Patients)

		Oral specimen	
	Absent	Positive	Negative
Splenomegaly	11	9	2
Hepatomegaly	5	2	3
Hepatosplenomegaly	13	10	3
Edema	14	11	3
Moderate to marked proteinuria	4	3	1
Complete syndrome of hepatospleno- megaly, proteinuria, and edema	16	13	3

sis of the kidneys. Similarly, in one patient (case 16) in whom the liver and spleen were so large as almost to fill the abdomen, the biopsy specimen contained but a minimal amount of amyloid, found only after a careful search.

In those cases which comprised group II, we did not have a single positive biopsy. These patients, as mentioned above, had no clinical evidence of amyloidosis, and the Congo red test, in each, had been negative. This experience supports the impression that a negative Congo red test, in the absence of positive clinical findings, usually means that generalized amyloidosis is not present. As will be shown below, however, a negative Congo red test, when other findings are present, has no such meaning.

The results of gingival biopsy in the third group, composed of cases in which amyloidosis was suspected but not proved, are perhaps most interesting of all. They show that biopsy of the gingivae may demonstrate amyloid when all other means fail to establish the diagnosis. The studies in these patients are summarized in Table III, but far more instructive and revealing is an analysis of several of the cases themselves.

TABLE III

Study of Oral Biopsy in Patients with Clinically Suspected but Unproved Generalized Amyloidosis

Case	Clinical examination		Edema	Proteinuria	Laboratory studies		Clinical diagnosis	Oral biopsy			Examination of other tissues
	Enlargement				Congo red absorption	Chemical examination of blood		Site	Result	Extent	
	Liver	Spleen									
40.*	+++	+	+++	++++	61, 57, 93, 59, 64	Hypoproteinemia	Amyloidosis (see text)	Mucobuccal fold	Positive	+++	Liver biopsy: amyloidosis, ++
41.	0	0	++	++++	31, 29, 41, 10	Hypoproteinemia, azotemia	Amyloidosis (see text)	Gingiva	Positive	+	
42.	++	+	+++	++++	40, 23, 50	Azotemia, no hypoproteinemia	Uncertain (see text)	Mucobuccal fold	Positive	++++	Necropsy: generalized amyloidosis
43.	+	0	0	++	31, 50, 59	Moderate azotemia	Glomerulonephritis	Mucobuccal fold	Negative		
44.	+++	+++	0	0	41	Normal	Uncertain; Pott's disease	Gingiva	Negative		Splenic biopsy; no amyloid
45.	++	0	+++	±	68, 50	Normal	Cardiac failure	Gingiva	Negative		Liver biopsy: congestion, no amyloid
46.	+++	0	+++	++++	26, 40	Hypoproteinemia, azotemia	Amyloidosis (see text)	Gingiva	Positive	++++	Liver biopsy: amyloidosis, ++
47.	0	0	++	++++	37, 33	Hypoproteinemia (slight)	Amyloidosis (see text)	Gingiva	Positive	+++	

* Cases 19 to 39 were in group II.

Case 40. J. L. was a white male, 34 years old, whose illness began in 1941. He was found to have bilateral fibrocaseous pulmonary tuberculosis and was admitted to Sea View Hospital in March, 1945. Besides his pulmonary tuberculosis he was found to have a nephrotic syndrome as well, with marked proteinuria, hypoproteinemia, hypo-albuminemia, and extensive edema, most marked in the lower limbs, sacrum, and scrotum but noted in all dependent parts. Since hepatosplenomegaly was present also, a provisional diagnosis of amyloidosis was made. A Congo red test done on August 1, 1945, resulted in 61 per cent absorption, which is not confirmatory of amyloidosis. The test was repeated on November 13, 1945, and 57 per cent absorption occurred. With these results, the diagnosis of amyloidosis could not be made with assurance. Meanwhile proteinuria continued, varying from 4 to 11 gm. per liter and from 7 to 25 gm. in 24 hours. Although the liver decreased in size from 7 to 3 cm. below the costal margin, the edema remained approximately the same and the serum albumin ranged from 1.58 to 2.52 gm. per cent. Fractionation of the urinary proteins showed a considerable amount of globulin, averaging about 30 per cent of the total. Hepatic and renal function tests yielded normal results. Since no other cause for the nephrotic syndrome could be found, it was still felt that renal amyloidosis was responsible. A third Congo red test on April 6, 1946, showed 93 per cent absorption. Since tests in this range are frequently unreliable,[7] biopsy was decided upon.

Biopsy of the mucobuccal fold was performed on May 10, 1946, and revealed fairly large deposits of amyloid, establishing the diagnosis and confirming the clinical impression. Liver biopsy by laparotomy showed amyloidosis in that organ as well.

Case 41. C. L. was a colored male, 31 years of age, who was discovered to have Pott's disease in 1939. A spinal fusion was performed in 1941 and, despite an apparently adequate result, abscesses formed in 1942. These were aspirated, leaving draining sinuses. There were no abnormal findings in this patient's urine for 4 years after the onset of his disease but in October, 1943, rather marked proteinuria appeared and persisted. The liver and spleen were not palpable, but a Congo red test was done to investigate the possibility that renal amyloidosis might underlie the proteinuria. On November 1, 1943, this test showed 70 per cent absorption, a result which is found both in patients with amyloidosis and in those without this complication. Other Congo red tests were "negative," yielding 31 per cent absorption on September 11, 1945, and 29 per cent absorption on March 12, 1946.

Pseudo-arthrosis being present, revision of the spinal fusion was performed on March 18, 1946. Several weeks later the proteinuria doubled in daily quantity, despite oliguria. Increased renal damage was evidenced by the development of moderate azotemia, with urea nitrogen reaching 60 mg. per cent and creatinine, 3.4 mg. per cent. Simultaneously, marked hypoproteinemia was noted, with serum proteins as low as 3.04 gm. per cent and serum albumin 1.33 gm. per cent, with concomitant generalized edema. At the onset of the period of clinical distress a fourth Congo red test was done with 45 per cent absorption. Thus, even though renal amyloidosis was suspected, it could not be proved by the Congo red test. Gingival biopsy was performed on May 25, 1946. Perivascular amyloid was present, and the diagnosis of renal amyloidosis was established. Congo red absorption on September 19, 1946, was 10 per cent.

Case 42. O. D. was a white male, 52 years old, who first showed symptoms of pulmonary tuberculosis in 1941, at which time he was admitted to Sea View Hospital. He was known to have diabetes and to have suffered from recurrent bouts of congestive cardiac failure since 1932. Electrocardiograms showed evidence of arteriosclerotic heart disease. His pulmonary tuberculosis progressed until 1944

but remained quiescent after that. During his hospitalization, he was noticed to have enlargement of the liver, proteinuria, and edema. All these could have been explained by cardiovascular disease, especially since there was no hypoproteinemia or hypo-albuminemia to account for the edema. However, particularly because of the rather severe proteinuria, which was greater than that usually found in renal arteriosclerosis, amyloidosis also was suspected. A Congo red test was done on August 6, 1945, and showed 50 per cent absorption, which is within normal range. During 1945 and 1946 the patient began to show signs of renal failure, with developing azotemia. This again could have been explained by either cardiovascular-renal disease or amyloidosis. Clinically, differential diagnosis was uncertain. On June 4, 1946, a specimen was taken from the mucobuccal fold. Abundant amyloid was found in the biopsy specimen and a diagnosis of generalized amyloidosis was made.

The patient died in uremia on August 15, 1946. Autopsy revealed generalized amyloidosis, with massive involvement of the kidneys, consistent with amyloid uremia.

Case 46. M. S. was a colored female laundry worker, 35 years old, who first developed pulmonary tuberculosis in 1939. Her disease, however, remained quiescent until 1943, when it became reactivated, causing her admission to Sea View Hospital in April, 1944, with caseous-pneumonic tuberculosis. Proteinuria was present on admission and continued during hospitalization. In March, 1945, her liver became palpable. A Congo red test on March 14, 1945, showed 26 per cent absorption and a diagnosis of amyloidosis could not be made. Proteinuria continued and reached 15 gm. in 24 hours with 50 per cent globulin on fractionation of the urinary proteins. In April, 1946, edema appeared, together with hypo-albuminemia. The Congo red test again was negative, showing, on April 9, 1946, only 40 per cent absorption. Gingival biopsy was then performed. Extensive amyloid deposits were present. A liver specimen obtained by laparotomy on May 16, 1946, showed moderate amyloidosis. This provided adequate explanation for the ensuing rise in the nonprotein nitrogen of the blood, the urea nitrogen being 60 mg. per cent on May 22, 1946. The patient died in uremia on June 6, 1946. Necropsy was refused.

Case 47. T. N. was a young Puerto Rican female, who was found to have end-stage caseous-pneumonic tuberculosis in July, 1945. On admission, her urine was normal and remained so until April, 1946, when, suddenly, there was found a 4 plus proteinuria. Although neither the liver nor spleen was palpable, amyloidosis was suspected and a Congo red test was done. It showed 37 per cent absorption on April 23, 1946. This, of course, did not confirm the diagnosis of amyloidosis. The proteinuria continued, averaging 8 gm. in 24 hours, with approximately 40 per cent globulin on fractionation of the protein present (suggestive of renal amyloidosis). In June, 1946, peripheral edema appeared. The Congo red test was again performed on October 2, 1946. It resulted in 33 per cent absorption. The liver and spleen were still not palpable. Gingival biopsy was done on October 23, 1946. Moderately extensive deposits of amyloid were found, and the diagnosis was established.

COMMENT

It is interesting to note that Congo red absorption, insufficient for a diagnosis of amyloidosis, was found in patients with considerable amyloid in the gingival specimens. It may be that faulty Congo red absorption can be explained by the presence of amyloid of a character

unsuited to chemical or physical affinity with Congo red. We feel, however, that a more likely explanation is simple quantitative insufficiency of the absorbing substance, amyloid. Of the 5 cases of amyloidosis with "negative" Congo red tests, 4 had extensive deposits of amyloid in the gingivae, while of the 14 with positive biopsies in the group with complete absorption, only 4 had heavy deposits. This is further support of the observation that there is no correlation between the amount of amyloid in the viscera and that in the gingiva. This peculiarity of distribution is no doubt responsible for our being able to demonstrate amyloid deposits in the gingivae of patients with what has heretofore been called "moderate amyloidosis." Diagnosis has always been difficult in these patients. In the original report introducing the Congo red test, Bennhold[5] reported a case which showed only 22 per cent absorption and yet was found to have splenic amyloidosis on post-mortem. He offered as an explanation the inability of small amounts of amyloid to absorb much Congo red. This deficiency of the Congo red test has plagued many workers in this field, since approximately 25 per cent of cases of amyloidosis have "negative" Congo red tests, in which but little Congo red is absorbed. Yet small amounts of amyloid does not mean innocuous amyloid, because there may be little amyloid in the body, yet that little may be concentrated in a vital organ, particularly the kidney, and lead to serious consequences. This was clearly the situation in case 46. Therefore, gingival biopsy, when positive, may well have particular value in cases of "moderate" amyloidosis, especially when the Congo red test is uncertain. The irregularity in the distribution of amyloid also limits the value of the gingival examination, as shown by the negative biopsies obtained in 4 of the 18 cases of clinically proved amyloidosis. Whether this is due to the complete absence of amyloid in the gingivae or whether the portion selected for study failed to contain deposits which were present in other areas is not yet known. That the latter explanation is probably the correct one is suggested by our experience that amyloid was often scattered in its distribution, with much of the tissue entirely free of it. In fact, in a number of specimens, it was present in only one limited site, usually perivascular, in a vessel-containing area of the epithelium. In one patient, case 16, we could find no amyloid in the first slide examined. However, in a second section, amyloid was clearly seen about a single blood vessel. Experiences such as this have led us to the conclusion that difficulty with the procedure may be minimized by examining several sections of the biopsy specimen and, in selected cases, taking biopsies from several sites.

Although the Congo red stain was often unsatisfactory, failing to

delineate clearly the amyloid from the fibrous tissue and hyaline structures, we found methyl violet guiltless in this respect, except when metachromatic artificial light or direct sunlight was used. No such difficulties were noticed with diffuse daylight. We did not encounter the irregularities of staining which have made interpretation of biopsy specimens in primary atypical amyloidosis frequently uncertain and which are, in fact, characteristic of that disease.[16,19,21] Similarly, the lack of variation in the methyl violet staining of the amyloid in our cases is consistent with previous experience in generalized amyloidosis, where irregularity of staining is uncommon. Too, in each of the 8 cases of positive gingival biopsy in which we had the opportunity of examining other tissues, the amyloid present therein did not vary in staining qualities from that in the gingiva.

We believe that gingival biopsy may find its principal sphere of usefulness in diagnosis of those cases of amyloidosis in which other methods fail, especially where the Congo red test is negative. In this connection, it should be remarked that mishaps have occurred following the administration of Congo red, [8,27] so that it might be wise, in some cases, to substitute gingival biopsy for repeated Congo red testing. Finally, gingival biopsy may serve one other useful function: to demonstrate, unequivocally, the presence of amyloidosis in cases in which surgical operation or other means of arrest or cure of the primary disease is contemplated, so that the possibility of regression of the amyloidosis might be studied. This use of biopsy has been employed previously by Waldenström[11] who, after subjecting his patients to repeated liver punctures, reported anatomic evidence of the regression of the disease in several cases. However, the use of serial gingival biopsy for this purpose is not without uncertainty, and sanguine expectations as to its value in the study of regression should be tempered.

SUMMARY AND CONCLUSIONS

1. The clinical diagnosis of generalized amyloidosis is often difficult for the characteristic triad of hepatosplenomegaly, proteinuria, and edema is present in less than 25 per cent of cases of proved amyloidosis. The Congo red test is a valuable diagnostic procedure but suffers from serious limitations: Apparent inability to demonstrate small or moderate amounts of amyloid, occasional false positives, and ill effects consequent upon the intravenous injection of the dye.

2. Biopsy, especially of the liver and spleen, has been employed both clinically and in experimental studies for the anatomic diagnosis of generalized amyloidosis. However, visceral biopsy, although valuable, is limited in its application. Biopsy of the oral tissues, particu-

larly the gingiva, has been investigated to learn whether it would be useful in the diagnosis of amyloidosis. The accessibility of the tissue, its resistance to infection and hemorrhage, and the uncomplicated simplicity and ease of the technic make the procedure inviting.

3. Forty-seven patients were studied. In 18 in whom the clinical diagnosis of amyloidosis had been made with reasonable certainty, amyloid was found in 14, upon gingival biopsy. There was no correlation between the amount in the gingiva and that in the viscera.

4. A negative gingival biopsy does not rule out amyloidosis. It is recommended that multiple sections of the specimen be examined and, if still negative in the face of strong clinical evidence of amyloidosis, that biopsy be repeated in another site.

5. Amyloid deposits were found in the gingivae of patients with but moderate amyloidosis, including those cases in which repeated Congo red tests were "negative." Heretofore, there has been no simple method by which to establish the diagnosis of amyloidosis in these patients.

REFERENCES

1. Auerbach, O., and Stemmermann, M. G. Surgery in the tuberculous patient with amyloidosis. *Am. Rev. Tuberc.*, 1946, 53, 333–344.

2. Rosenblatt, M. B. Amyloidosis and amyloid nephrosis. *Am. J. M. Sc.*, 1933, 186, 558–567.

3. Campuzano, L. L., Cardenas, C. N., Rodriguez, J. J. M., and Ledon, C. Degeneracion amiloidea y tuberculosis. *Rev. de Tuberc., Havana*, 1941, 5, 102–105.

4. Altnow, H. O., Van Winkle, C. C., Maly, H. W., and Williams, L. E. Renal amyloidosis. Clinical course and pathologic lesions in sixteen cases. *Arch. Int. Med.*, 1935, 56, 944–975.

5. Bennhold, H. Über die Ausscheidung intravenös einverleibten Kongorotes bei den verschiedensten Erkrankungen insbesondere bei Amyloidosis. *Deutsches Arch. f. klin. Med.*, 1923, 142, 32–46.

6. Stemmermann, M. G., and Auerbach, O. The value and limitations of the Congo red test for amyloidosis. *Am. J. M. Sc.*, 1944, 208, 305–309.

7. Selikoff, I. J. Diagnosis of generalized amyloidosis by the Congo red test: definitive diagnostic criteria. *Am. J. M. Sc.*, 1947, 213, 719–727.

8. Selikoff, I. J., and Bernstein, I. J. Systemic reactions to the intravenous administration of Congo red. *Quart. Bull., Sea View Hosp.*, 1946, 8, 131–141.

9. Selikoff, I. J. The significance and interpretation of the "doubtful" Congo red test. *Quart. Bull., Sea View Hosp.*, 1946, 8, 194–204.

10. Josefson, A. Cited by Waldenström.[11]

11. Waldenström, H. On the formation and disappearance of amyloid in man. *Acta chir. Scandinav.*, 1928, 63, 479–530.

12. Dantchakow, W. Über die Entwicklung und Resorption experimentell erzeugter Amyloidsubstanz in den Speicheldrüsen von Kaninchen. *Virchows Arch. f. path. Anat.*, 1907, 187, 1–34.

13. Morgenstern, Z. Zur Frage über Amyloidose und Resorption. *Virchows Arch. f. path. Anat.*, 1926, 259, 698–725.

14. Dick, G. F., and Leiter, L. Some factors in the development, localization and reabsorption of experimental amyloidosis in the rabbit. *Am. J. Path.*, 1941, 17, 741–753.

15. Nomland, R. Localized (lichen) amyloidosis of the skin. *Arch. Dermat. & Syph.*, 1936, 33, 85–98.

16. Gottron, H. Systematisierte Haut-Muskel-Amyloidose unter dem Bilde eines Skleroderma amyloidosum. *Arch. f. Dermat. u. Syph.*, 1932, 166, 584–615.

17. Königstein, H. Über Amyloidose der Haut. *Arch. f. Dermat. u. Syph.*, 1924–25, 148, 330–383.

18. Golden, A. Primary systemic amyloidosis of the alimentary tract. *Arch. Int. Med.*, 1945, 75, 413–416.

19. Weber, F. P., Cade, S., Stott, A. W., and Pulvertaft, R. J. V. Systematized atypical amyloidosis with macroglossia. *Quart. J. Med.*, 1937, n.s. 6, 181–193.

20. Koletsky, S., and Stecher, R. M. Primary systemic amyloidosis. Involvement of cardiac valves, joints and bones, with pathologic fracture of the femur. *Arch. Path.*, 1939, 27, 267–288.

21. Michelson, H. E., and Lynch, F. W. Systematized amyloidosis of the skin and muscles. *Arch. Dermat. & Syph.*, 1934, 29, 805–820.

22. Warren, S. Generalized amyloidosis of the muscular systems. *Am. J. Path.*, 1930, 6, 161–167.

23. Reimann, H. A., Koucky, R. F., and Eklund, C. M. Primary amyloidosis limited to tissue of mesodermal origin. *Am. J. Path.*, 1935, 11, 977–988.

24. Lewis, T. Pain. The Macmillan Co., New York, 1942, p. 42.

25. Taran, A. A further improved Congo red test for amyloidosis. *J. Lab. & Clin. Med.*, 1936–37, 22, 975–977.

26. American Trudeau Society, report of the committee on standard laboratory procedure: minimum laboratory standards. *Am. Rev. Tuberc.*, 1942, 45, 103–114.

27. Macht, D. I., Harden, W. C., and Grumbein, M. L. Toxicological studies on Congo red. *J. Am. Pharm. A.*, 1939, 28, 495–499.

INDEX TO VOLUME XXIII

INDEX OF SUBJECTS

* Abstract of paper presented at the meeting of the American Association of Pathologists and Bacteriologists held at the University of Illinois, Chicago, May 16 and 17, 1947.

INDEX OF AUTHORS

* Abstract of paper presented at the meeting of the American Association of Pathologists and Bacteriologists held at the University of Illinois, Chicago, May 16 and 17, 1947.